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VOL. XXXIX—No. 3

Issued January 1, 1916

BALTIMORE, U. S. A.

1916

Entered as second class matter, August 13, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 39 JANUARY 1, 1916 No. 3

INITIAL LENGTH—INITIAL TENSION AND TONE OF AURICULAR MUSCLE IN RELATION TO MYO AND CARDIODYNAMICS

ROBERT A. GESELL

From the Laboratory of Physiology, Washington University Medical School, St. Louis

Received for publication November 2, 1915

In previous experiments (1) I showed the beneficial effect of auricular systole on ventricular output, and ascribed this effect to various factors. Among other factors, were increased initial length of ventricular fiber and increased intraventricular tension produced by auricular systole.

A study of the relative importance of these two factors was the object of the present research.

It is well known that the conditions under which a muscle contracts, e.g., load, inertia, amount and rate of shortening, affect materially the amount of heat, tension and work developed. Differences of interpretation of fundamental factors concerned in muscular contraction make the literature difficult of review. Only such work as bears directly on this problem will be cited.

Emphasis has been given to both initial length and initial tension as important factors determining the nature of contraction. Blix (2) makes a clear distinction between initial length and initial tension, emphasizing the importance of length of fiber to muscular contraction. If we accept the suggestions of Blix, much of the work done on myodynamics must receive a new interpretation, for it is evident that such factors as load, inertia, initial tension, etc., determine to a great extent the length of fiber at any given moment before and during contraction. But in the work of Schwann (3), Blix (4), Hill (5) and others in which length is regarded as the important factor, initial length of fiber was not varied independently of initial tension: the muscle requiring active

stretching to produce the desired lengthening. Vice versa, initial tension was not increased without an accompanying increase of initial length.

Experiments permitting independent variation of either initial length or initial tension would be valuable in determining the relative importance of each of these factors. The auricle of the turtle through its great and gradual distensibility, and its rhythmic oscillations of tone make such experiments possible.

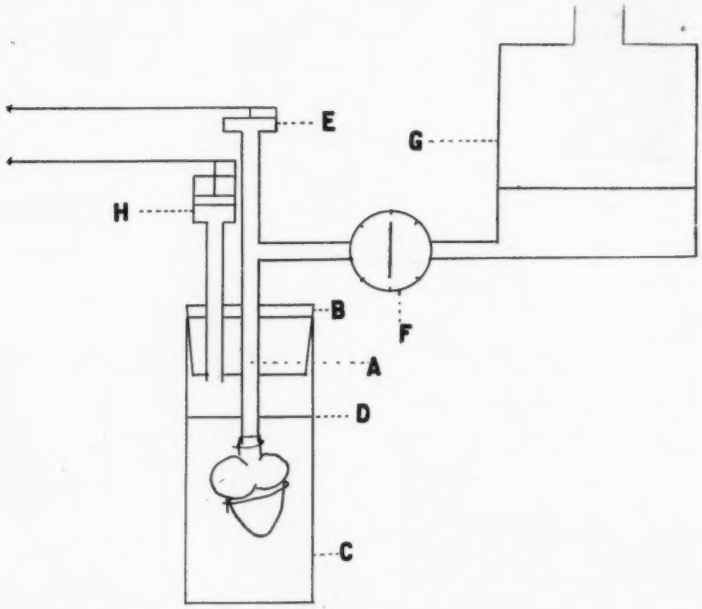

Fig. 1

These auricles show two types of contractions, rapid clonic superimposed upon slow tonic contractions. Bottazzi attributes the slow tonic contractions to the sarcoplasm and the rapid clonic to the fibrillae. Although the results of this research do not definitely prove that such is the case, they point in that direction, and therefore, Bottazzi's suggestions are used in places as a working hypothesis.

Figure 1 shows the method employed. With the heart in situ the left Cuvierian duct, the hepatic vein and the auricular ventricular

junction are ligated. A large cannula is passed through the right Cuvierian duct well into the right auricle and securely tied. The heart is then removed and the cannula connected with tube (*a*) which passes through the rubber stopper (*b*). Tube (*c*) is filled with Ringer's solution to the level (*d*) and tightly stoppered. The right auricle connects directly with a delicate membrane manometer (*e*) and indirectly through valve (*f*) with a large diameter bottle (*g*). The manometer, tubing and valves are filled with Ringer's solution to the exclusion of air, and the bottle filled to the level shown.

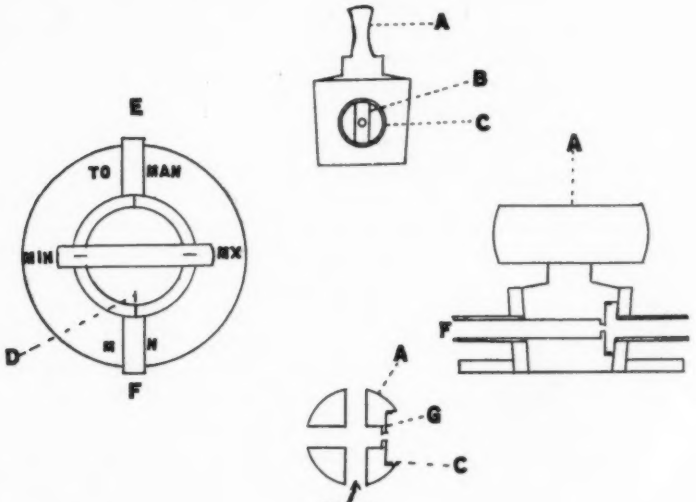

Fig. 2

The auricular filling pressure and the resistance to auricular contraction are largely regulated by varying the height of the bottle. The tension developed by auricular contraction is recorded by manometer (*e*). With valve (*f*) open the solution is free to pass to and from the bottle with each contraction and relaxation of the auricles—changing the level (*d*) in tube (*c*). These displacements of solution are recorded with the piston recorder (*h*). Time is marked in seconds and fifths of seconds.

Both isotonic and isometric contractions were recorded. They were obtained by manipulating valve (*f*). This valve is supplied with a gold beater's skin. Change in the position of the cock therefore

regulates rate and direction of flow through it. Since the valve will serve the more common purpose of a maximum, minimum and mean valve for the registration of pressures it will be described as such. Its construction is shown in figure 2. It consists of three portions, cock, socket, and base. The cock, which is freely removable, has two holes running through it at right angles to each other. One is free and of uniform bore. The other is enlarged at one end, fitted with a centrally perforated platform (*g*). A strip of gold beaters skin is held in place over the center of the platform by a thin brass ring (*c*). The socket is supplied with two tubes (*e*) and (*f*). Tube (*e*) marked "To Man" leads to the manometer. Tube (*f*) connects with the source of pressure. Marks on the socket mx, min, and mean indicate the position of the cock. By rotating the cock to these marks maximum, minimum or mean pressure may be recorded.

The isotonic contractions were not purely isotonic, due to the inertia of the solution, neither were the isometric contractions purely isometric, due to the capacity of the manometer. However, the two types of contractions were distinctly different and had their special values.

With the valve open the solution passes to and from the auricle with each auricular cycle, and socalled isotonic contractions are obtained. In these contractions work primarily is performed (see figs. 3, *a* to *b* and 6). The effects of variation of initial length of fiber on these contractions were studied by the use of two methods. (1) Keeping the tension constant and waiting for a spontaneous rhythmical change in length of fiber accompanying auricular tonus oscillation (see fig. 3, *a* to *b*). (2) Sudden increase of auricular filling tension, allowing this rise of tension to progressively lengthen the auricular fiber (see fig. 6). The latter method was also used to determine the effect of tension.

With the valve completely closed the solution cannot move and the auricle contracts isometrically with constant initial and final length. In these contractions tension primarily is developed (see figs. 3, *b* to *c* and 10) and the effect of initial tension may be studied in auricles showing oscillations of tone, for with every tonic contraction and relaxation there is a corresponding rise and fall of tension. The effect of length on isometric contractions may be studied by placing the valve at maximum position, permitting solution to enter, but not to leave the auricle. In such cases a series of isometric contractions with gradually increasing initial length of fiber and constant initial tension are obtained.

In all the records, with the exception of figure 11, the upper tracing

represents volume changes; the middle, tension changes; and the lower time in seconds.

In the volume tracings down stroke represents auricular emptying, up stroke auricular filling. This tracing is an index to volume output, volume, length of fiber, and condition of tone. The upper points indicate the initial volume and initial length of fiber; the lower points, final volume and final length of fiber. In the tension tracing up stroke represents rise of tension, down stroke fall of tension. The upper points of the tension curves represent final tension, the lower, initial or filling tension.

The results of this research were obtained from 27 experiments on the auricle of the turtle. (Pseudemys elegans.) In these experiments some of the auricles were free from tonus oscillations while others showed them to varying degrees.

The property of auriclar tone alone made this research possible. Naturally a method dependent on auricular tone, giving data concerning the relation of length and tension to muscular contraction, must also throw some light on the mechanism of tone. The nature of auricular tone will therefore be discussed.

RESULTS

1. Effect of increase of initial length of fiber on isometric contractions of auricles showing marked oscillations of tone (see figs. 3 and 4).

In figure 3 both isotonic and isometric contractions are recorded: isotonic from *a–b*, isometric from *b–c*. The volume output per beat, the tonus oscillations and length of fiber are represented in the upper tracing. A complete tonus oscillation occurs between points *a* and *b*. At *b* the auricle is at its smallest and is ready to undergo tonic relaxation. Here the valve is turned to maximum position, allowing Ringer's solution to enter but not leave the auricle. A series of isometric contractions follow in which the initial length of fiber gradually increases while the initial tension remains constant. Accompanying the increase in volume (length of fiber), there is progressive increase of final tension.

In figure 4 the effects of increased length of fiber are more marked for a special reason. In that case the auricle is practically empty at the moment the valve is turned while in figure 3 the auricle is fairly well filled. Owing to a more rapid increase in the volume than in the surface of a growing sphere it is evident that the relative as well as the absolute increase in length of fiber is greater in figure 4, than in figure 3.

In addition to the increased final tension note that the duration of maintenance of any given tension also increases with the length of fiber. The importance of this factor in cardiodynamics is strengthened by the fact that the maintenance of the higher tensions is increased relatively more than that of the lower tension.[1]

2. Effect of increase of initial length of fiber on isometric contractions of auricles showing no oscillations of tone, or, at most, weak oscillations.

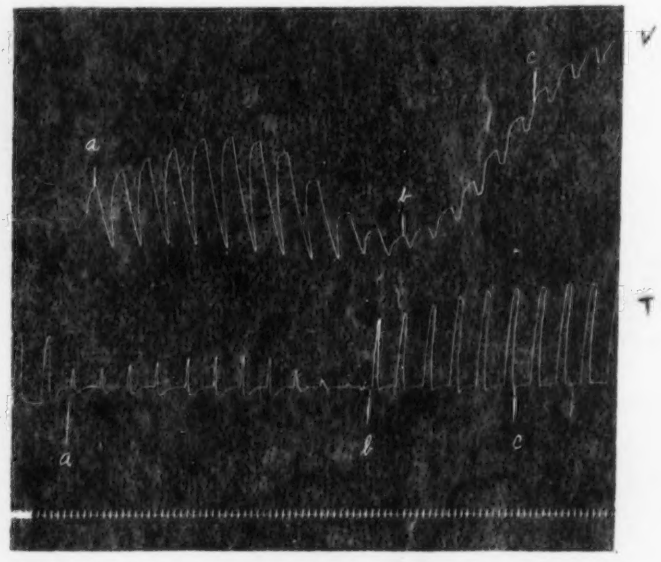

Fig. 3

From the results shown in figures 3 and 4 it might well be argued that the importance of length of fiber was not fully established; that other factors may have entered affecting the efficiency of muscular

[1] A secondary crest appears in some of the figures, e.g., figure 4. This crest is increased in prominence with increased initial length and initial tension. Guenther (20) describes two contractions in heart muscle which might account for tension curves shown in figure 4. In addition the abundant literature (6–19) on the nature of the secondary crest of the contraction curve of striated muscle suggested that the auricular contraction might be composed of a rapid and a slow contraction but the possibility of extraneous liquid oscillations accounting for the second crest does not justify definite conclusions at present.

contraction, for in these records a spontaneous internal condition of the muscle arose permitting for a time the lengthening of the fiber although the filling tension remained constant. This changing internal condition permitting the dilatation of the auricle might also affect the force of contraction, in some unknown way. To determine whether length of the fiber is the factor of prime importance records were taken from auricles which were entirely free from tonus oscillations, or showed them to a small extent only. To obtain a series of isometric contrac-

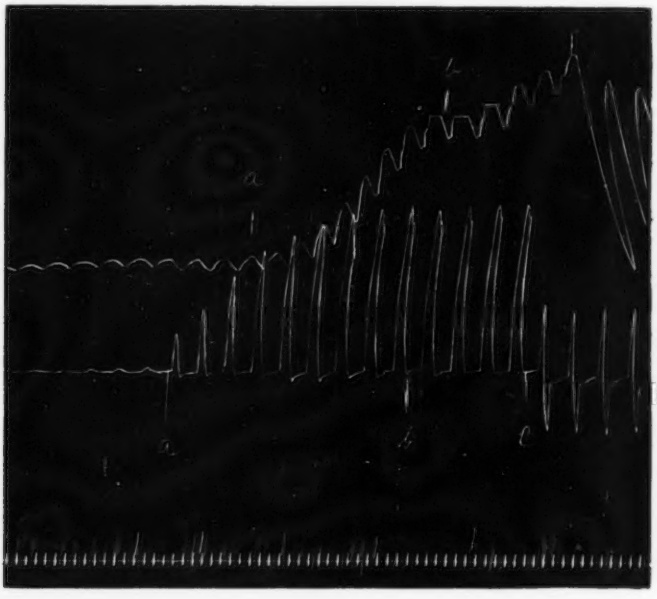

Fig. 4

tions with progressively increasing length of fiber, in such auricles, the valve was placed at maximum position. This usually sufficed to produce a dilatation of the auricle. If it did not, the filling tension was slightly increased, when the desired lengthening invariably followed.

The results obtained show that the increased efficiency of auricular contraction accompanying an increase of length of auricular fiber, is substantially the same—whether the fiber spontaneously increases in

length by some altered internal condition, or is increased in length by some external force. This would indicate that length is the important factor.

According to Bottazzi's (21) theory of tonus oscillation, and Blix's (22) theory of muscular contraction, the varying efficiency accompanying tonus changes can be ascribed to a mechanical factor, a lengthening and shortening of the fibrillae by the contraction and relaxation of the sarcoplasm. Whether this is the only explanation is better studied on auricles contracting under isometric conditions, during a tonus oscillation. Under these conditions, the variation of the important factor—length—is eliminated; the expenditure of energy is concentrated in development of tension alone rather than tension and work and, therefore, variations of auricular energy exchanges are better gauged.

3. The relation of initial length of fiber to isotonic contractions of auricles showing tonus oscillations (see figs. 3 and 5).

In figure 3 it will be noticed that the volume output varies directly as the initial volume of the auricle, and that the final tension developed follows an almost parallel course. To interpret the changes in volume output and final tension we must bear in mind a number of factors. The long muscle is more efficient than the short. The volume of the auricle increases as the cube, its surface as the square of the radius. Therefore, the greater the volume of the auricle the greater will be the volume change per unit length of shortening of auricular fiber. Constant initial tension from a mechanical point of view, should have no varying effect on the final tension developed. Inertia of the Ringer's solution affects final tension to an extent dependent on the strength and quickness of contraction and amount of solution to be displaced per unit of time.

The usual relation of length of fiber to solution moved, and tension developed is shown in figure 3.

Occasional exceptions to this rule occurred, and suggest that tonus oscillations are of a more complicated nature than usually considered, and that auricular muscle may strikingly regulate its efficiency by ways other than change in length of fiber. Figure 5 is an example of such an exception. In this record the crests and troughs of the tonus oscillations shown on the upper tracing are marked and set off on the tension tracing below. On the upper tracing two pairs of corresponding points—indicating the same length of fiber—are marked off. Such points, 1 and 2, and, 3 and 4 mark contractions occurring during different phases of tonic oscillation; 3 during the period of tonic relaxation

Fig. 5

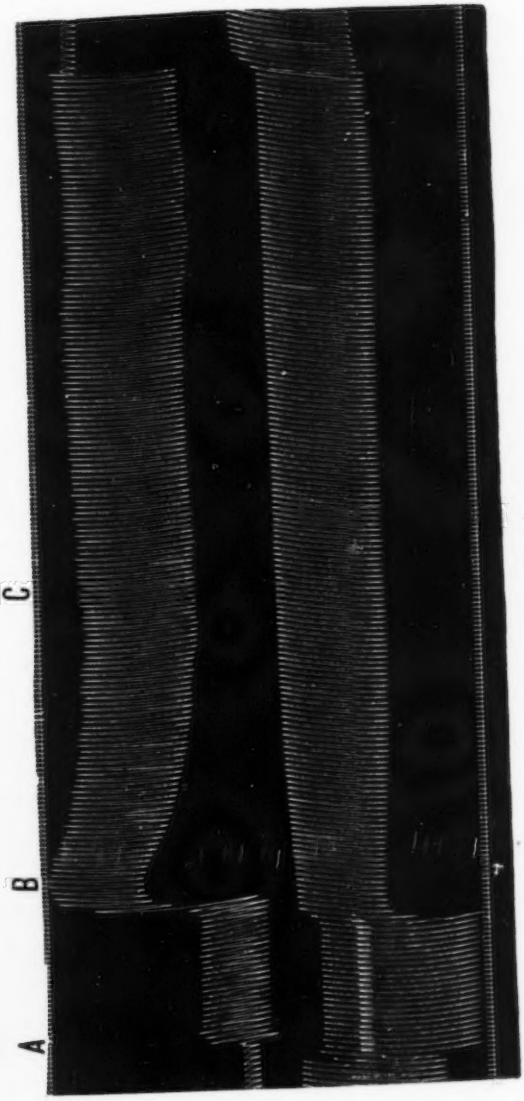

Fig. 6

and 4 during the period of tonic shortening. The amplitude, quickness of contraction, and tension developed are greater at 3 than at 4; this difference occurring although the length of fiber and initial tension are the same in both cases.

1 and 2 mark contractions occurring during the same phase of tonic shortening. The volume and tension changes are considerably different.

Figure 5 shows that changes other than variation of length produce marked differences of efficiency of muscle. Two possibilities, namely changing coordination of contraction, or block are suggested in explanation of this case.

4. The effect of initial length on isotonic contractions in auricles showing no or small tonus oscillations (see fig. 6).

If the filling tension of an auricle contracting isotonically is suddenly increased the auricle suddenly grows in volume and from that moment its activities follow a definite course. The sudden large increase of volume gives place to a more gradual increase, and this in turn to a gradual decrease in volume. The relation of volume output and final tension to these changes of initial length of fiber is a special point of interest. In figure 6 the filling tension is increased from 12 mm. to 31 mm. of water. The rapid increase of length of fiber is at once accompanied by an increased volume output and final tension. During the gradual increase in length of fiber the volume output does not increase. After the length of fiber has reached its maximum, final tension and volume output increase, even though the length of fiber decreases.

Since the length of fiber decreases and final tension and output increase, this record indicates a specific enhancing effect of tension on muscular contraction. This effect may be due to increased auricular tone, which usually results from increased filling tension. As will be pointed out tonic force is an important factor in myodynamics.

Experiments showed that the amplitude of volume output and tension developed under isotonic conditions were not reliable indices of the power of the auricle to develop tension under isometric conditions. Therefore, experiments similar to that represented in figure 6 were performed in a slightly modified way (see fig. 7). The initial tension was increased and the auricle allowed for the most part to contract under isotonic conditions. At selected intervals, as the length of fiber decreased, a few isometric contractions were recorded, care being taken to throw in the valve at the end of the period of relaxation. It will be noticed again that as the length of fiber gradually decreases the tension

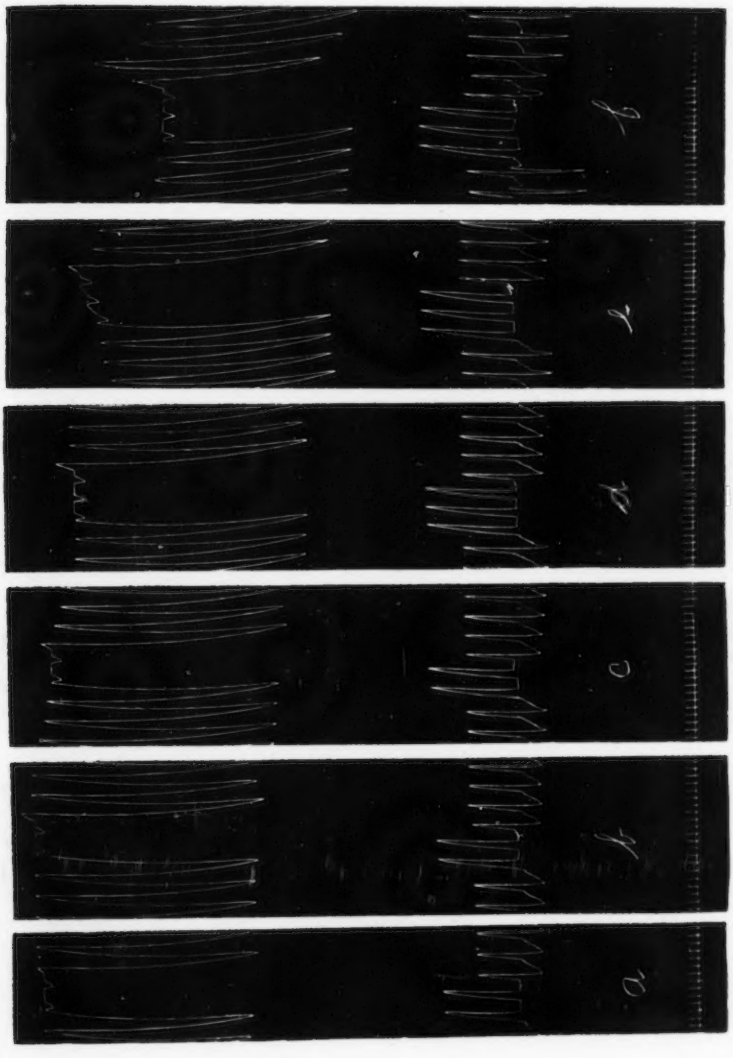

Fig. 7.

developed in both isotonic and isometric contractions, progressively increases up to point *F*. The volume output bears a sl ghtly different relation to the changing length of fiber. It reaches its maximum at *E*, then suddenly decreases to a volume output less than that obtaining at *A*. The relation of vo ume output, initial length of fiber and final tension developed at *F* are of particular interest.

The initial length of fiber at *F* is considerably shorter than at *E* and here the volume of solution moved no longer continues to increase as the initial length decreases. The final tension developed at *F* under isotonic conditions is less than at *E*, but the force of contraction as represented by the isometric contraction is greater. In connection

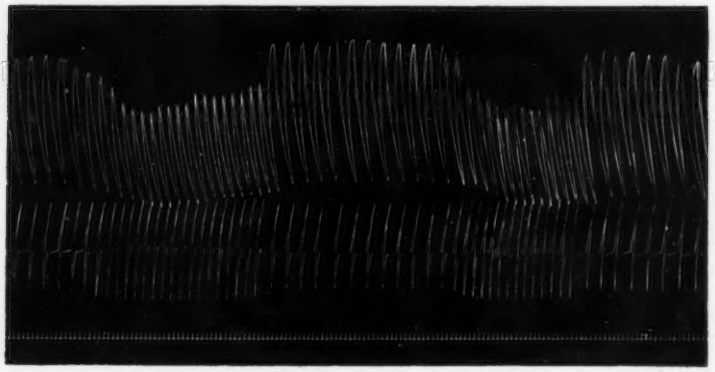

Fig. 8

with these facts it is of interest to note the shape of the volume curves, the sharp angles at the end of relaxation and beginning of contraction from *A* to *E* compared with the rounded curve at *F*, illustrating the importance of tonic force in muscular contraction.

To illustrate again the inverse relation between length of fiber and force of contraction, a chance record different from figures 6 and 7 is described (see fig. 8). In this record groups of frequent contractions alternated with groups of less frequent contractions. When the rapid groups occurred the auricular fiber shortened just as the mammalian ventricular fiber shortens when its sequence is increased. Though the auricular fiber was considerably shortened, the efficiency of the contractions as measured by the tension developed was noticeably increased. Whether the energy liberated was greater or not is

difficult to say, but the record does give evidence that one phase of muscular contraction, namely, quickness, varied inversely as the length of fiber.

5. The relation of simultaneous change in both length and initial tension to the work performed and tension developed by auricular contraction (see fig. 9).

Recording tension, length and volume changes under isotonic conditions, starting with high initial tension and lowering this tension by steps, interesting results concerning tonus, final tension and optimum conditions for work are obtained. The results of such an experiment are shown in figure 9 and Table I.

With each diminution of initial tension the length of the auricular fiber diminishes accompanied by changes in final tension and volume output. The volume output first increases (from *a* to *c*). then decreases (*c* to *e*). Along with these changes a peculiar relationship between the difference in initial lengths and the difference in final lengths of fiber in two adjacent tracings occurred. For instance, between tracing *A* and *B*, and *B* and *C* the difference between the final lengths is great. Between *C* and *D*, and *D* and *E* just the reverse relation obtains. The point of reversal comes just at a time when the effects of tone show on the form of the volume curves. At *A*, and *B*, both contraction and relaxation begin and end abruptly at *C* tonus oscillations are faintly visible. Beyond the point of reversal the end of contraction passes gradually into relaxation. Relaxation is slowed and the base of the relaxation curve is considerably rounded. At this point tonus oscillations appear. The final tension developed falls with every decrease of initial tension but not in the same proportion, as is seen by comparing the calculated tension with the actual tension. The calculated tension of *D* equals the difference between the initial

TABLE I

GROUP OF CONTRACTION	INITIAL TENSION IN MM. OF WATER	FINAL TENSION IN MM. OF WATER	CALCULATED FINAL TENSION IN MM. OF WATER	TENSION DEVELOPED IN MM. OF WATER	RATIO OF DECREASE OF INITIAL LENGTH TO DECREASE OF FINAL LENGTH	VOLUME OUTPUT IN CC.
A.....	81	97	104	16	a/b–7/18	1.00
B.....	60	83	90	23	b/c–8/16	1.55
C.....	30	60	61	30	c/d–19/8	1.74
D.....	17	48	47	31	d/e–16/5	1.42
E.....	10	40		30		1.00

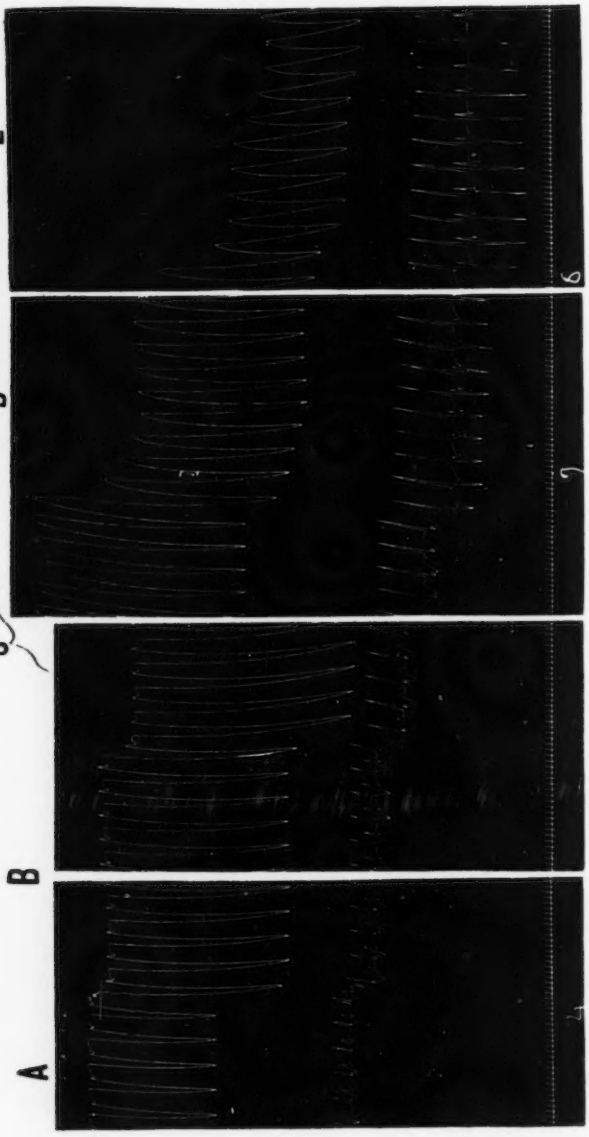

Fig. 9

tension of *D* and *E* plus the developed tension of *E*. For *C* and *D* the calculated tensions correspond closely; for *B* and *A* they fall short. This change like others mentioned occurs at the point of reversal and was ascribed in part to a more or less economic storage of potential energy and resistance to filling tension, these in turn being attributed to the increased relative importance of the tonic force. The volume changes have a somewhat similar explanation: that the volume depends upon a balance of forces—the filling tension working against the elasticity of the muscle (connective tissue and fibrillae), and the changing elastic force of tonus (sarcoplasm). Which group of contractions represents condition favoring maximum efficiency of auricular contraction is difficult to decide. Comparing the differences of tension developed with each contraction and the amount of fluid moved, one would place it at *C*, the point of reversal. At *C* the tonus oscillations are faintly visible. The auricle, therefore, is filled to its maximum without harmful stretching of its fibrillar elements, the sarcoplasmic contractions presumably protecting the fibrillae against harmful stretch. This assumption is made in the light of Bottazzi's theory of tone. In addition, the muscle at *C* is probably more elastic than at *A* and *B* and therefore gives up more perfectly with each contraction its stored potential energy represented by the initial filling tension. Conditions at *D* and *E*, however, are safer for here the muscle is even more elastic. The fibers are shorter and the tonus oscillations more marked. Therefore, among the factors of increased safety may be considered: (1) Greater reserve adaptation at the disposal of the muscle by simple increase in length of fiber; (2) more economic resistance to filling tension; (3) more economic storage of potential energy.

6. Relation of initial tension to isometric auricular contractions of constant initial length of fiber. (See fig. 10.)

If valve *F* is completely closed while the auricle is showing energetic tonus oscillations, the solution cannot move to or from the heart and the length of fiber must necessarily remain constant.

With each tonic contraction and relaxation there is a corresponding rise and fall of initial tension.

The relation of initial to final tension was not constant. But usually, an increase of initial tension led to an increase of final tension. The quantitative relation between the increase of initial and final tension is of special significance. There are indications, which were previously discussed, that initial tension may be stored in the muscle as potential energy. On contraction of the fibrillae this potential energy may be

added to the force generated in the fibrillae. If the increase of final tension is just equal to the increase of initial tension, the effect of initial tension may be purely mechanical and may have no specific effect on the processes underlying contraction. But in figure 10 the increase of final tension is considerably greater than the corresponding increase of initial tension and therefore, cannot be accounted for entirely by the storage of initial tension as potential energy. Some other factor comes into play affecting the processes of contraction proper. What this factor is, is difficult to determine, owing to the fact that the variations of initial tension are the results of tonus oscillations. The interpretation of the records depends largely upon our conception

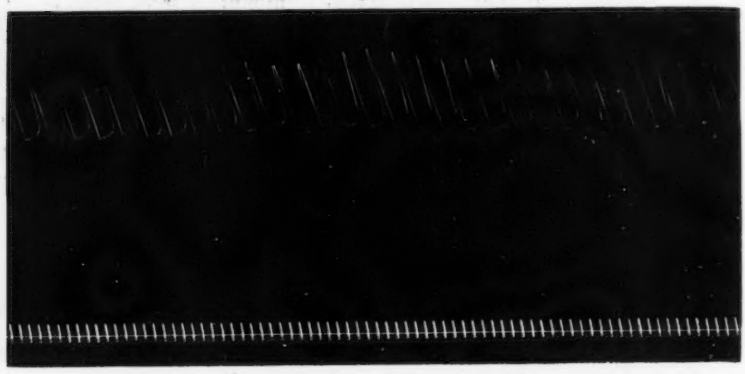

Fig. 10

of the nature of tone. For instance, would the force of contraction be the same in figure 10 if the processes underlying tonus oscillations were not accompanied by oscillations of initial tension? Figure 5 may, in part, answer this question. From 1 to 5 the auricle is contracting isotonically, from 5 to 7 isometrically with constant initial length of fiber. The relation of length of fiber to strength of contraction in this record was previously discussed. From 6 to 7 the relation of final to initial tension is exactly the reverse of that shown in figure 10. During the period of high initial tension the final tension is actually less than that developed during a period of relatively low initial tension. From 5 to 6 the initial tension remains constant yet the final tension increases. Some factor other than initial tension is responsible for

the fluctuation of final tension, and the question is, do such records prevent us from drawing conclusions as to the effect of initial tension on isometric contraction as seen in figure 10? In answer to this question it can be said that such records as figure 5 are the exceptions; that increase of initial tension is usually accompanied by increase of final tension; that contrary to figure 5, the work and tension developed by an isotonically contracting auricle varies as the length of auricular fiber, whether that length is increased through tonus oscillations or by external force. In figure 5 muscular contractions seem to be affected by oscillations of tone, whether changes of initial tension occur or not. Compare the isotonic record with the isometric. The decreased final tension in both cases invariably accompanies the phase of tonic shortening. The decreased final tension is accompanied by a slower and longer contraction possibly indicating decreased coordination, interference with conduction. If the tonic shortening is always accompanied by the tendency to slower, and longer maintained contraction, through disturbed conduction as suggested for figure 5, the effects of initial tension noted in figure 10 become more significant.

7. *Development of tone.* Oscillations of tone are of frequent occurrence in apparently normal auricles. Some auricles show only small oscillation or are completely free of them. In these auricles the general tone level can be changed by varying the filling tension as shown in figure 6. Here the sequence of length changes, following an increase of initial tension will be recalled: first, a rapid increase; second, a slower increase followed by a decrease in length of fiber. This sequence, as well as the accompanying changes of volume output and final tension were ascribed in part to the development of tone. The influence of tension on tone can be shown in a reverse manner. If the filling tension is decreased in an auricle in which the tonus has been previously increased, the increased tonus shows itself by the auricle contracting down until the cavity is completely obliterated. The auricle remains in this condition for some time, indicating that the tonic force of the auricular muscle is greater than the filling tension; soon the tonus decreases, as evidenced by the gradual increase of auricular volume.

Isotonic contractions can be changed to isometric by closing the valve completely. If the valve is closed at the end of diastole the fiber will be relatively long and the initial auricular tension equal to the filling tension. If the valve is closed at the end of auricular systole, the fiber will be short and initial auricular tension less than the filling tension. In either case the tonus diminishes.

In the first case of isometric contractions, there is continuous tension on the auricular wall, but the active stretch (change in length of fiber) present in the isotonic contraction is missing. This may be the important factor for the development of tone.

8. Regulation of length of auricular fiber. An auricle, free from tonus oscillations, contracting isotonically with a constant filling tension maintains a constant volume. If the valve is turned to maximum position the volume of the auricle may or may not change depending upon the condition of tonus (see fig. 11). This figure shows auricular volume changes from two different experiments. If the auricle has a low general tone level (without oscillations) the volume increases promptly on turning the valve to maximum position (fig. 11x). An auricle of higher tone level (also without oscillation) shows no volume change on turning the valve. If such an auricle shows tonus oscillations as in figure 11 *y*, the volume changes depend upon the moment the valve is turned: if turned at *f*, at the beginning of tonic contraction, no change in volume occurs; if turned at *g*, the volume increases, the curve running parallel to the initial volume curve occurring in an isotonic record (*e–f*). These records bring up the question of the regulation of length of auricular fiber. The two types of behavior on subjection to conditions mentioned indicate that both muscular elements, fibrillae and sarcoplasm, may be factors, varying in importance depending on the relative strength of the tonic force.

Figure 11 *x*, was obtained from an auricle in which no tonus oscillations were apparent. The tone of the muscle was low. The behavior of the auricle in this case suggests that the fibrillae were the length determining elements, for after isometric conditions are established the auricular fiber cannot shorten; but at end of systole, if the fibrillae have no internal support they may relax and be distended by the filling tension. In the case of figure 11 *x*, the amount of solution entering the auricle with each diastole is approximately the same for six relaxations, and then gradually diminishes which compares with a distensibility curve of an imperfectly elastic substance.

If the sarcoplasm is the length determining element, owing to the property of maintaining a continued contraction, we should expect, if there are no tonus oscillations and the tonic force is equal to the filling force, no change in volume; if tonus oscillations are present a record corresponding to figure 11 *y*.

The records seem to indicate that the two elements, fibrillae and sarcoplasm, work together, regulating the gradual and the diastolic

volume changes; the response to distention depending on the relative importance of the two factors. Where the tonic force is weak, as in figure 11 *x*, a constant volume is maintained only while the auricle contracts isotonically, when contracting isometrically (*c* to *d*) the tonic force is not sufficient to maintain the isotonic volume without the assistance of the resistance to stretching which the isotonically contracting heart offers during each diastole. If the tonic force is strong

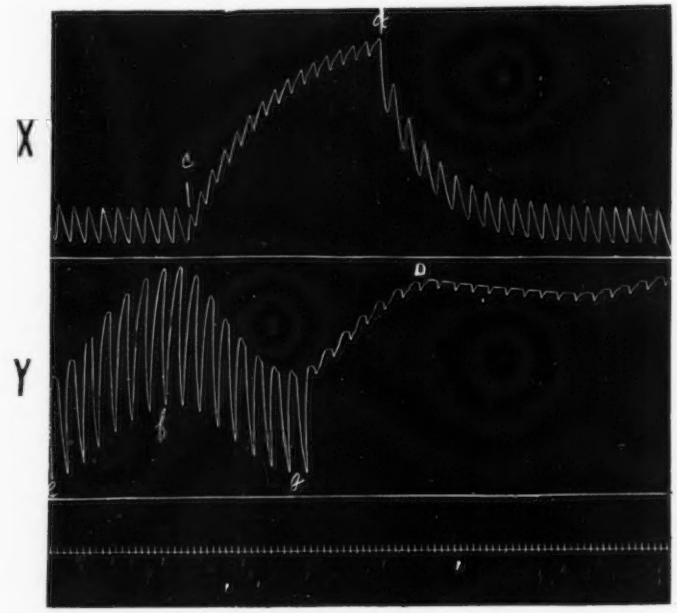

Fig. 11

it is sufficient by its own force to prevent lengthening of the fibers under isometric conditions.

8 B. Relation of rate of contraction to length of fiber. Tonus oscillations may occur in auricles in which the fundamental contractions are suppressed by drugs or eliminated by cutting away the sinus, giving evidence of the relative independence of the two rhythms. It is interesting however, in connection with regulation of length of fiber to note whether there is any relation between rate of contraction and

length of fiber. The relation in the mammalian heart is known: increase in rate producing a decreased length of ventricular fiber. In the turtle's auricle this relation to a certain extent is true, indicating that the rate of relaxation of fibrillae is too slow to maintain the volume obtaining at a slower rate of contraction (see fig. 8). Records were obtained in which groups of frequent contractions alternated with groups of less frequent contraction. In some of these records tonus oscillations occurred. Groups of frequent and less frequent contractions occurred in the various phases of tonic oscillations, but apparently had no effect on the amplitude of the oscillations.

DISCUSSION

The relation of initial length and tension to final tension developed

Both initial length and initial tension have been considered important factors determining the nature of muscular contraction. The relative importance of these factors owing to the difficulty of varying one factor independently of the other, is hard to determine.

Schwann (23) 1873, states that the amount of tension developed by muscular contraction varies directly as the length of the muscle. Blix (24) 1895–1902, finds that the longer the muscle fiber at the moment of excitation, the greater the amount of work, tension and heat developed; the normal extended length of muscle in situ being the most efficient. Employing improved methods of recording heat development, Hill (25) arrives at similar conclusions. In a later study on the frogs sartorious, Evans and Hill (26) find the development of heat and tension to increase on extending the muscle 12 per cent of its normal resting length. While writing this paper the work of Kozawa (27) comes to my attention. Employing methods resembling those used in the present research, he studied the effects of variation of length of the turtle's ventricular fiber in relation to ventricular contraction and found the force of contraction to vary directly as the length of fiber.

In all of the work cited, as far as I am able to determine, increased initial length was accompanied by increased initial tension. Increased initial tension was necessary to produce the desired increase of initial length.

Patterson, Piper and Starling (28) in their paper "On the Regulation of the Heart Beat" show figures in which initial length of ventricular fiber varied, initial intraventricular tension remaining constant. The final tension developed by ventricular systole varied directly as the

length of fiber. These figures they consider "are of special importance since they enable us to differentiate between changes in tension and changes in length as factors determining the amount of energy set free in the contraction of heart muscle." Length of fiber rather than tension was considered the important factor.

Straub (29) dealing with the principles of regulation of heart beat employed the same methods but did not obtain such results. He found initial length and initial tension always to vary together and considers initial tension as the important factor determining the efficiency of ventricular systole.

Since both length of fiber and initial tension have been regarded as important factors in myodynamics the question of clearly differentiating them is important. In view of this fact, and of the fact that the contemporaneous work of Straub and Patterson, Piper, and Starling on the heart are not in agreement, the work reported in this paper was performed.

Turtle's auricular muscle, through its special properties, permits large variations of length of fiber—initial tension remaining constant. Within these limits the ability of auricular muscle to develop tension varies as the length of fiber, agreeing with the conclusions of Schwann, Blix, Hill, and others. This relation holds despite the fact that an increasing surface maintains the increased final tension.

This relation of initial length to final tension suggested to Blix his theory of "chemically active surfaces" as explanatory of his conclusions. According to this theory, the energy exchanges occurring during muscular contraction are dependent on the amount of exposed surface of the active contractile elements. The fibrillae are considered the active elements, their external surface the important surface. Increase in the length of the muscle fiber increases the amount of fibrillar surface exposed and therefore the development of energy. Decrease in length has exactly the opposite effect.

The importance of this conception is evident, and if accepted certain theories of muscular contraction must be changed, for the theory considers the longitudinal surface of the fibrillae as the surface at which the processes responsible for muscular contraction occur. Theories similar to Roafs (30) in which cross sectional areas are considered important surfaces might require modification.

Tension

The relation of tension to muscular contraction is more difficult to explain. It is known that variations of initial tension or variation of resistance to the muscle during contraction affect materially the nature of contraction. Hill has shown that increase of initial tension and interference with muscular contraction increase the energy exchanges. This increase is in part accounted for by the tendency of both factors to increase the initial length or retard the shortening of the muscle during the first part of the contraction, the period in which the energy exchanges for contraction are determined.

But specific effects of tension other than the influence on length of fiber are indicated in the present research. What is the nature of this influence? Can it be explained on a chemical, physical, or mechanical basis, or must we revert to the theory of secondary excitation?

The effect is partly mechanical. The increased initial tension, whether produced by an external force or by an isometric tonic contraction, is stored as potential energy. When the quick clonic contraction occurs this potential energy is given up and added to the energy developed by the clonic contraction. Hill makes the same suggestion for striated muscle.

The mechanical storage of energy is not sufficient in all cases to explain entirely the effects of initial tension (see fig. 10. a series of isometric contractions of constant initial length, but oscillating initial tension). The final tension changes are greater than the corresponding changes of initial tension. The possibility of the tonic condition of the muscle underlying the variation of inititial tension, affecting the final tension was pointed out in connection with figure 5. Other instances of an effect of tension were cited in auricles contracting under isotonic conditions (see fig. 6. The effect of sudden increase of initial tension). From *B–C*, the final tension and the work performed progressively increase though the initial length of fiber decreases. In this instance the rate of auricular rhythm is slightly increased by the change of initial tension. Therefore it might be argued that with the increase in rate, the muscular metabolism may be so altered as to give the Treppe phenomenon. But it seems just as plausible to argue that if the function of automaticity is augmented the function of contractility might at the same time be affected. There is no question but that tonus is increased, and, as has been pointed out, this would increase the efficiency of muscular contraction. If one contractile element (sarcoplasm)

is affected by tension the possibility of the other being influenced cannot be dismissed.

In connection with this suggestion comes the difficulty of knowing definitely in any given condition whether it is the fibrillae or the sarcoplasm which bears the tension. During rest when the tone of the auricular muscle is well developed the sarcoplasm probably bears most of the tension. Sarcoplasm is the nutrient medium of the fibrillae; therefore if the external surface of the fibrillae is the important "chemically active surface," and if the sarcoplasm is altered by change in initial tension as evidenced by the accompanying variations of tonus, the possibility of initial tension having an indirect effect on the fibrillae is indicated.

Nature of Tonus Oscillations

Oscillations of auricular tone were first described by Fano and his associates (31 and 32). They describe two auricular rhythms, a rapid fundamental and a slow undulatory contraction, and conclude that the two rhythms are processes of two distinct elements of the same muscle fiber.

Bottazzi (33) localizes the fundamental contraction in the fibrillae, the tonic contraction in the sarcoplasm, pointing out a direct relation between the intensity of tonus oscillations and the amount of sarcoplasm present.

Botazzi and Grünbaum (34) show the resemblance between sarcoplasmic oscillations of tone and the contraction of smooth muscle, finding the agents or conditions which inhibit or augment auricular tonus oscillations to have an analogous action on smooth muscle.

Bottazzi (35) also points to a direct relation between the prominence of the second crest of contraction of striated muscle and the amount of sarcoplasm present. He finds conditions influencing the prominence of the second crest to have a similar effect on smooth muscle contraction. The absence of rhythmical tonus oscillations in striated muscle he ascribes to the lost property of automaticity.

Mosso (36) however, states that tonus oscillations occur in the respiratory muscles of man.

In striated muscle Grützner (37) allots to the white fibers the function of rapid contraction and to the red fibers slow contraction, which regulates the length of the muscle giving inner support and smoothness to the contraction of the white fibers.

Rosenzweig (38) finding smooth muscle cells in the auricular wall

of the turtle, attributed the tonus oscillations to the smooth muscle and the rapid clonic contraction to the heart muscle proper.

The question relative to the myogenic or neurogenic origin of tonus oscillations is of interest.

Pugliese (39) is of the opinion that the automatic movements of the frog's stomach are initiated by and depend on the integrity of Auerbach's pluexus.

Fano states that sections of the turtles auricle entirely free from nervous structures show oscillations of tone. Bottazzi, though believing that sarcoplasm is independently rhythmical, finds the oscillations influenced by vagus or sympathetic stimulation. All workers are agreed that these nerves have an effect. The variation of results of Fano, Bottazzi, Rosenzweig and Oinuma, as Oinuma (40) pointed out, are due to the variations of intensity of tonus oscillations and to the difficulty of isolating for stimulation pure bundles of vagus and sympathetic fibers.

The double innervation of striated muscle described by Perroncito (41), Boeke (42) and others is of interest in this connection, though at present it has not been established for auricular muscle. Perroncito finds in addition to the myelinated fibers, extremely fine nonmyelinated fibers making connection with the motor end plate and other parts of the muscle. No suggestion of the function of these fibers is offered but in a later communication (43) he states that these fibers come from the sympathetic system. Mosso (44) recorded oscillations of tone in the respiratory muscles of man and applied the finding of Perroncito suggesting that striated muscle has a double functional innervation—one for the quick movements, the other for tonic movements; the oscillations of tone being initiated and controlled by cells in the sympathetic nervous system.

Brown's (45) results on gradual compression of nerve support the theory of double functional innervation.

S. de Boer (46) modified Brondgeest's tonus experiment on the frog. Instead of cutting the sciatic plexus he cut the sympathetic fibers going to the plexus and obtained equal decrease of tone. He considers the myelinated fibers as controlling the fibrillae and the nonmyelinated from the sympathetic system as innervating the sarcoplasm. S. de Boer (47) however, was unsuccessful in eliciting only the first contraction of veratrinized muscle on stimulating a nerve bundle free from sympathetic fibers. He, nevertheless, believes that the second contraction is sarcoplasmic, and that the failure to get only the single

quick contractions was due to the stimulation of the sarcoplasm by the muscles' own action current.

The results of this research, while not throwing any definite light on the place of initiation of oscillations of tone and of their nervous control, indicate that these oscillations are a result of sarcoplasmic contraction and relaxation. If the fibrillae easily adapt their length as they seem to do, contraction and relaxation of the sarcoplasm should respectively shorten and lengthen the fibrillae. Then, according to Blix's theory, the sarcoplasm should, by varying the length of the fibrillae, control the efficiency of the muscle. If the efficiency of muscle is altered in such a simple fashion, then it must naturally follow that an increase in length of fiber whether that occurs during a tonus oscillation or is caused by increased filling tension should have substantially the same effect. This is the case.

Though in the main the oscillations of tone may be looked upon as simple contraction and relaxation of sarcoplasm affecting the efficiency of muscular contraction only in a mechanical way, certain results of this research indicate that oscillations of tone influence the muscular efficiency in other ways. Figure 5 is a case in point. The significance of these records has been discussed. Bottazzi's (48) description of two kinds of tonus oscillations is also important in this connection. In one kind, the amplitude of the fundamental contraction remains constant regardless of the change in length of fiber; in the other the amplitude varies directly as the length. The study of Fano and Fayod (49) on the relation of electrical variation in length of fiber is of interest in the same connection.

Figure 5 is important in regard to Rosenzweig's theory of auricular tonus oscillations. It cannot be brought into line with his theory for it is difficult to see how contraction of smooth muscle cells by increasing the initial tension should decrease the final tension.

Development of Tone

Muscular tone is considered a property of normal muscle. But it has been suggested that oscillations of tone in the auricle of the turtle are a pathological manifestation of poorly nourished muscle. Fano states that tonus oscillations occur in normal animals with the circulation intact. It has been my experience that the hearts showing greatest energy also show the largest tonus oscillations and the greatest tendency to develop tone when subjected to conditions which tend to increase tone.

Stretching or increased filling tension was the one stimulus which almost invariably produced an increased auricular tone. The irregularity of rate of tonus oscillation prevented me from determining whether increased tension increased the number of oscillations of tone per unit of time as it did the rapid clonic contractions. One point came out clearly: a definite tension, for each auricle studied produced the largest tonus oscillations. The size of the oscillation is probably dependent on the degree of disturbed balance between force of filling tension and the force of tonic contraction.

An increased filling tension, whether the auricle is contracting isotonically or isometrically almost invariably produces increased tone. Under isotonic conditions the development of tone manifests itself by the shortening of the muscle fiber subsequent to the initial increase in length; under isometric conditions by an increase of intra-auricular initial tension. If stretching stimulates production of tone, does it do so directly as a mechanical stimulus or by electrical variations? Sherrington (50) considers stretching to be the adequate stimulus for nerve endings in such round viscera as the ductus choledocus and ureter. Lewandowsky and Einthoven (51) noted an electrical variation in the vagus on distending the lungs with artificial respiration. Ewald (52) found that stretching the closing muscle of the mussel produced an electrical variation similar to, but in the opposite direction to that produced by normal contraction. These references show that stretching of the auricle may well act as either a mechanical or an electrical stimulus for it has been suggested that a negative variation of a contracting muscle may restimulate that muscle to contraction (53). But in view of the fact that an electrical variation produced by stretching is slow in development and therefore of low stimulating power (54) as compared with the negative variations produced by the rapid contractions and that rapid contractions are not able to maintain the tone of muscle contracting isometrically, it seems that tension exerts its stimulus in another way. It has also been suggested (55) that rising contractile tension, if the sarcoplasm be very irritable, may be of stimulating value. But again, the rising tension occurring during isometric contraction of constant initial length fails to maintain a constant tone.

It seems most plausible that increased tension acts as the adequate stimulus to either the muscle fibers or the nerve endings in them by mechanical stretching. If data of double innervation of auricular muscle were at hand, the latter would seem more probable.

SUMMARY

The relation of initial length, initial tension, and tone of auricular muscle to myo- and cardiodynamics was studied.

Records of auricular tension and auricular volume were made with the auricle contracting under isometric and isotonic conditions.

A method permitting variation of either initial length or initial tension keeping respectively initial tension and initial length constant is described. In connection with this method a maximum, minimum and mean valve was devised.

With initial tension constant the strength and duration of contraction varies directly as the length of auricular fiber.

Increase of initial tension while the auricle contracts isotonically produces a sudden increase in volume which in turn gives way to a gradual decrease. This gradual decrease in volume is accompanied by increasing final tension and volume output, indicating a specific enhancing effect of tension on muscular contraction.

The same enhancing effect of tension is indicated, but not proved in isometric contractions with constant initial length, in which initial tension varies as a result of tonic contraction and relaxation.

The effects of initial tension may be due in part to its storage in the muscle as potential energy and later liberation during contraction.

In addition tension may have a specific effect on the process underlying contraction.

Tonic force is an important factor in auriculo-myodynamics of the turtle.

Both fibrillae and sarcoplasm may be initial length determining elements.

The increased efficiency of auricular contraction accompanying increased length of fiber is substantially the same whether the increased length is artificially produced or occurs spontaneously at the onset of a tonic relaxation—indicating that the varied efficiency of auricular contraction accompanying tonus oscillations is primarily due to a mechanical factor, namely, change of fibrillar length by contraction and relaxation of the sarcoplasm.

There are indications however, that tonus oscillations may affect fibrillar efficiency in other ways.

Tension, or stretching, is the adequate stimulus for the production of tone.

In regard to the relation of auricular systole to ventricular efficiency,

the results show the properties of auricular muscle to be such as to assure good ventricular filling.

Granted that auricular systole increases ventricular filling, the importance of auricular systole is evident.

By increasing the length of ventricular fiber, auricular systole increases the strength and duration of ventricular contraction.

By increasing the intraventricular tension, it increases the strength of ventricular contraction by adding its own force of contraction as potential energy stored in the ventricular walls.

The presystolic tension arising from auricular systole may have a beneficial effect upon ventricular contraction.

By increasing ventricular filling auricular systole brings about an enhancing surface volume relation. Since the surface of a sphere varies as the square of the radius and the volume as the cube, it follows (if the ventricles are considered roughly spherical) that the larger the ventricular volume the greater the ventricular output per unit shortening of muscle.

When this surface volume relation is considered in connection with the increased efficiency accompanying increased length of fiber and increased initial tension the beneficial effects of auricular systole are all the more evident.

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CONCERNING THE ACTION OF VARIOUS PITUITARY EXTRACTS UPON THE ISOLATED INTESTINAL LOOP

V. N. SHAMOFF, M.D.

PETROGRAD, RUSSIA

From the Laboratory of Surgical Research, the Harvard Medical School

Received for publication November 4, 1915

In their biological reactions the extractives of the adrenal medulla and of the posterior lobe of the hypophysis have many points of resemblance, and when tests are made for minute quantities such as may be present in the circulating blood, their differentiation may be difficult.[1] They both produce a haemodynamic response of some variability, but a pressor action is the dominant feature with a coincident slowing of the pulse rate; they both have a midriatic property on the isolated frog's eye; both are capable of provoking glycosuria and diuresis; both accelerate the coagulation of the blood; both have a specific action on the smooth muscle fibers of the intestine and uterus.

Those who are sufficiently familiar with the two substances can readily distinguish the haemodynamic reactions of one from the other; and adrenalin is more active in producing glycosuria just as pituitary preparations are more potent diuretics. But the most distinctive difference in the actions of the two substances has been supposed to lie in the relaxing effect of adrenalin on the unstriped muscle of the intestine, whereas posterior lobe extracts are commonly believed to uniformly

[1] In Dr. Cushing's monograph on the Pituitary Body and its Disorders (4) the following note occurs: "The similarity of the reactions of adrenalin and posterior lobe extract and the fact that they are both presumably present in the blood stream should make pause those who are accustomed to attribute to adrenalin alone the presence in the blood of a substance which has pressor qualities and which acts on unstriped muscle fiber. It is of interest that J. M. O'Connor (Ueber Adrenalinbestimmung im Blute, München. med. Wchnschr. 1911, lviii, 1439) has noted that the action of blood serum is greater on the rabbit's uterus than is the action of an epinephrin solution of the same concentration: and further, that while epinephrin inhibits the intestine, blood serum containing an equal amount of this substance stimulates it. He suggests that some other substance with similar properties must be present. In all likelihood it may prove to be infundibulin."

contract these fibers as well as those of the bladder and uterus. This action on the isolated intestinal loop is regarded by Hoskins (7) as the most sensitive and specific of all epinephrin reactions.

There are some references in the literature which are more or less contradictory to Hoskins's (7) statement that "no other gland shares with the adrenals their depressing effect upon the gut." Dale (5) in 1909 observed that "an isolated loop of intestine, the rhythm and tone of

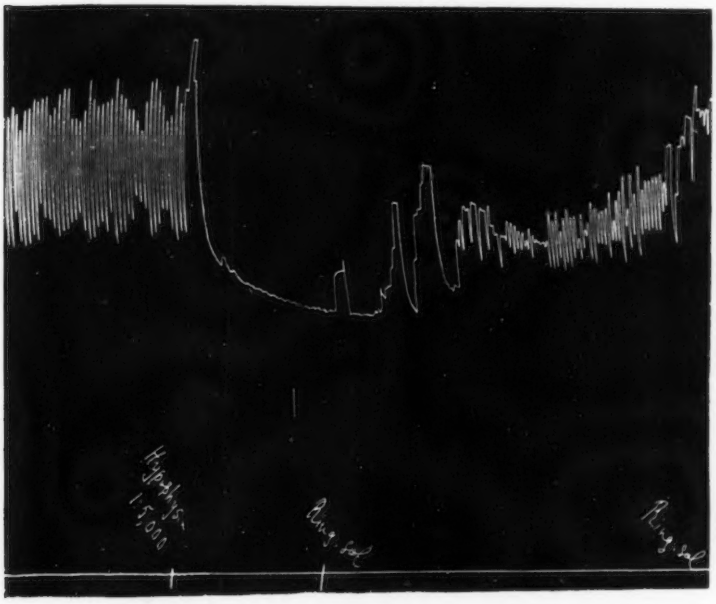

Fig. 1. Relaxing effect on isolated intestinal loop of 1 to 5000 dilution of hypophysin.

which are immediately inhibited by adrenalin, contracts though but feebly when pituitary extract is added to the bath." He supposes therefore that "the inhibition seen under normal conditions of circulation is due to the intense anaemia, which the vasoconstrictor action of the extract produces," and is led to believe "that the characteristic action of the extracts of the posterior lobe of the pituitary body is stimulation of plain muscle fibers." In the same year Foderá and

Pittaŭ (6) noted that the injections produced defecation, and Bell (2), who studied the influence of intravenous injections of pituitary extract upon different organs, observed strong peristaltic movements in the intestinal tract of rabbits and for this reason recommended the clinical use of pituitary preparations in intestinal stasis. Ott and Scott (8), in 1911 registered peristaltic movements by a balloon inserted in the intestine, and also found that infundibular extracts introduced into

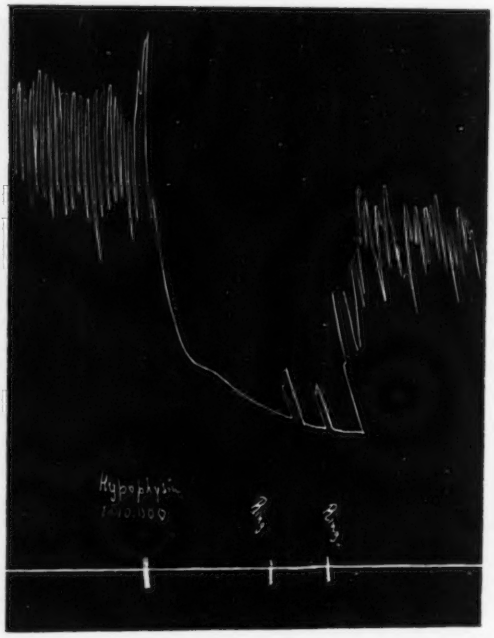

Fig. 2. Relaxing effect of 1 to 10,000 Hypophysin.

the circulation increased the extent of the intestinal contractions. Bayer and Peter (1) alone of all the authors we have consulted noted in 1911 that "pituitrin" (Parke, Davis & Co.) and fresh extract of the pituitary body might inhibit the movements and tone of the rabbit's isolated intestinal loop. As Schäfer and Vincent (10) showed to be true of the haemodynamic responses, so also these authors conclude that they are dealing with two substances, one soluble and the other

insoluble in alcohol, the former stimulating, the latter inhibiting, the muscle.

In the course of some experiments undertaken in this laboratory we desired, if possible, to find a delicate reaction for small amounts of posterior lobe substance in the circulating fluids, and under the presumption that the smooth muscle response was the most likely to fulfill our purposes, a series of preliminary tests were made with a variety of

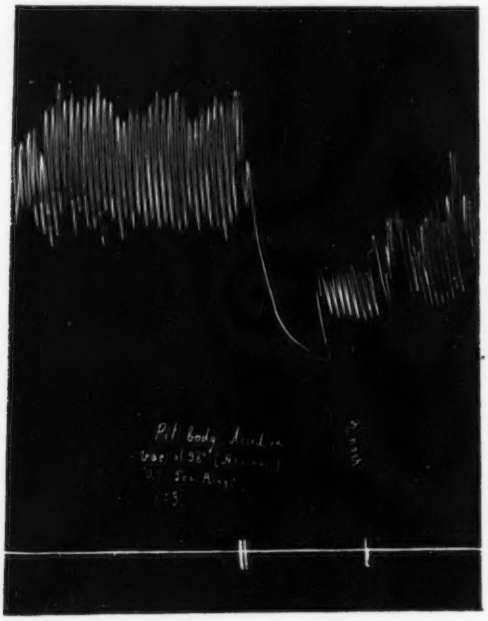

Fig. 3. Relaxing effect of posterior lobe extract dried in vacuo at 38° (Armour).

extracts which were in our possession. In the case of the isolated intestinal loop, it was found, as will be seen, that the effect of these preparations was most variable and inasmuch as reactions supposed to be characteristic of adrenalin were often obtained, this note has been prepared as a warning lest too great reliance be placed on this method as exclusively applicable to adrenalin.

The preparations employed were the following three proprietary fluid preparations: 1, "Pituitary Liquid" (Armour & Co.[2]); 2, "Pitui-

[2] Each cc. represents 0.2 gram of the fresh bovine posterior lobe.

tary Extract" ("Schering"[3]); 3, "Hypophysin" (Meister Lucius & Brüning[4]): also the following three dried extracts of posterior lobe which were taken up in Ringer's solution; 4, "Pituitrin A" (Parke, Davis & Co.[5]); 5, dried anterior lobe preparation (Parke, Davis & Co.);

Fig. 4. Reaction from 1 mg. of dried posterior lobe substance extracted with 5 cc. Ringer's solution.

[3] Each cc. corresponds to 3 grains of fresh glandular substance of the infundibular lobe.

[4] This preparation contains the four active substances of the eight substances isolated by Führer (Deutsch. med. Wchnschr., 1913, No. 11, p. 491) from the posterior lobe.

[5] This preparation had just been issued by the firm and a sample was placed in our hands. Of the original fluid pituitrin (Parke, Davis & Co.), which had been used by Bayer and Peter, no tests were made as no specimen was in the laboratory at the time. Nor did we test the infundin of Burroughs, Wellcome & Co., or the pituiglandol of the Hoffman-La Roche Co.

6, posterior lobe extract dried in vacuo at 38° (Armour & Co.); 7, dried preparation of (a) Pars anterior, (b) Pars intermedia, and (c) Pars nervosa, the standard preparations of this laboratory from sheep and oxen; and finally, 8, fresh extractives of bovine Pars anterior and Pars nervosa.

Fig. 5

Fig. 6

Fig. 5. The sediment further extracted with 5 cc. Ringer's solution, producing relaxation.

Fig. 6. The third extract of the sediment with 5 cc. Ringer's solution, producing slight relaxation.

The technique described by Hoskins was employed. The segment of intestine from 2 to 2.5 cm. in length was taken from a rabbit anaesthetized by urethan (in 1.5 gram per kilo weight) and suspended in actively oxygenated Ringer's solution at a constant temperature of 38°. The Ringer's solution was drawn off and replaced by the test fluids, kept at an equal temperature by immersion in the outer bath.

For purposes of control, various adrenalin dilutions were employed and they invariably produced the typical relaxation and inhibition of rhythmic contractions described by others.

The various pituitary preparations showed great variability in their response. The preparation which most constantly elicited the reaction supposedly typical of adrenalin, was "Hypophysin" which in dilutions of 1: 5000 (fig. 1) and 1: 10,000 (fig. 2) gave immediate inhibition of rhythmic movements and sharp relaxation of the isolated loop. The former regular movements and tone were promptly restored on replacing the bath with one of Ringer's solution.

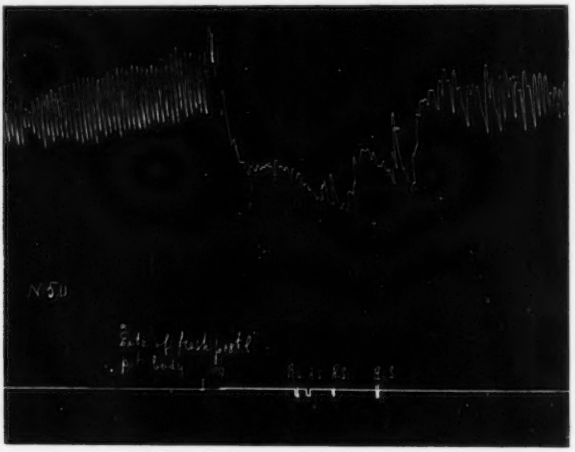

Fig. 7. Relaxing effect of freshly prepared posterior lobe extract 1: 100.

The second most potent substance, in this same direction, was the Armour extract of posterior lobe dried in vacuo at 38° (fig. 3). The powdered extract dissolves incompletely in Ringer's solution, but it apparently acts in weak dilutions. The three succeeding records were obtained from solutions made as follows. One mg. of the dried extract was shaken up in 5 cc. of Ringer's solution and after settling, the supernatant fluid was used for the test (fig. 4). To the precipitate another 5 cc. of Ringer's solution was then added with an equally definite response (fig. 5). Again to the second precipitate a third 5 cc. was added with almost as striking a relaxation as the preceding one (fig. 6) and this was repeated several times.

Fig. 9. Response from old dried preparations of posterior lobe substance. Relaxation following contraction.

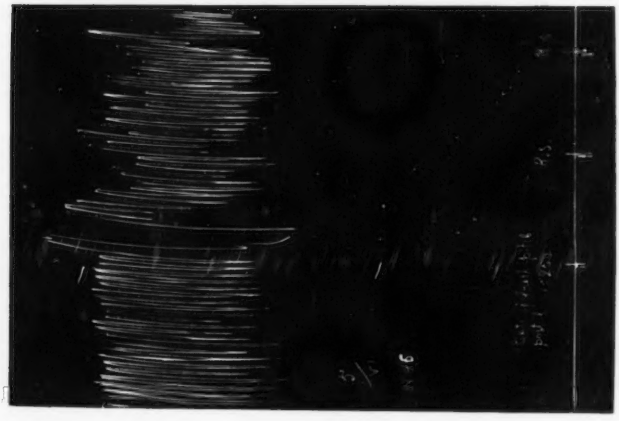

Fig. 8. Momentary relaxation from an extract of fresh posterior lobe 1:250.

Freshly prepared posterior lobe extracts made by grinding up the glandular substance with sand were, of our various preparations, the next in their inhibiting tendency on the loop, though some rhythmic contractions might persist during the relaxation (fig. 7) or the total period of relaxation might be brief and incomplete (fig. 8). Some dried laboratory preparations a year old gave some delayed responses occasionally with a primary contraction (fig. 9) and subsequent relax-

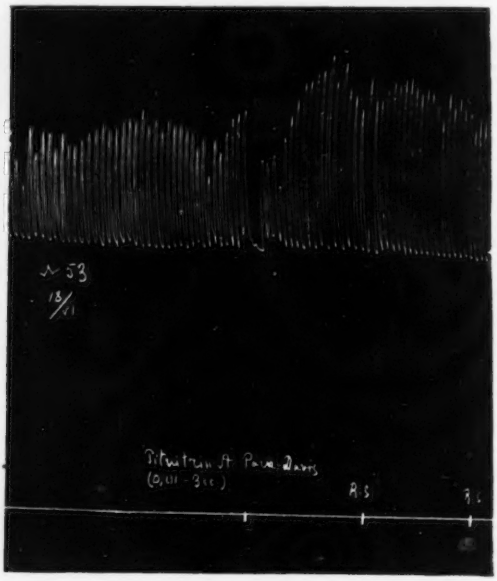

Fig. 10. Slight relaxation from "Pituitrin A" resembling the response (fig. 8) of freshly prepared extract.

ation with incomplete cessation of rhythmicity. "Pituitrin A" also usually gave a suggestive response (fig. 10), though it was incomplete and without definite relaxation, the response resembling that of the fresh posterior lobe extract (fig. 8).

The other posterior lobe preparations (e.g., Armour's "Liquid" and "Schering's" Extract) all gave a more or less outspoken reaction in the reverse direction, namely with an increase of tonicity and activity of peristalsis. Our laboratory preparations of Pars anterior and Pars

intermedia made from the same glands which furnished the posterior lobe extracts were entirely inactive and exerted no influence on the loop.

An effort was made to determine whether in correspondence with their variable action on the intestinal loop, the substances showed any differences as regards their blood pressure and diuretic reactions, but there was nothing conclusive about the responses and even those preparations which had no effect on the isolated loop gave typical haemodynamic responses. Cramer (3) expresses the opinion that the substances which act upon the pupil are not identical with these which stimulate the renal secretion and that the former are not only more delicate but are more easily destroyed, and it is quite probable that in the ordinary preparations we are dealing with more than one basal substance. The wide variability in the physiological activity of commercial pituitary extracts has been emphasized by Roth (9) and though crystalline bodies have been isolated by Houssay, Führer, Baudouin, Aldrich and others with tentative claims as to their representing the active principle or principles of the gland, they have not been generally accepted as the "active principle" in the sense that epinephrin represents the active principle of the adrenal medulla.

In view of the foregoing observations, it may be stated that there exists in the posterior lobe of the pituitary body some substance which has an action on the isolated intestinal loop resembling the action of adrenalin, a substance moreover which may be other than that which raises blood pressure and causes diuresis. The substance is not constant in the extractives prepared in the usual ways and may indeed be inconstant in the fresh glands from which these substances are prepared. The most definite relaxations were obtained from "Hypophysin" and from Armour's preparation made by drying the extract of the gland in vacuo at body temperature. Its presence or absence in the extracts may depend on the method of preparation and more accurate studies can only be made when this is perfected or when the active principle or principles of the gland become known and are capable of synthesis.

As the matter stands, it may be concluded that certain posterior lobe preparations are capable of producing relaxation of the isolated intestinal loop and of inhibiting its rhythmic contractions, resembling in this respect the extracts of the adrenal medulla.

I desire to express here my gratitude to Professor Cushing for the privilege of working under his guidance.

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ON THE SECRETORY DISCHARGE OF THE PITUITARY BODY PRODUCED BY STIMULATION OF THE SUPERIOR CERVICAL SYMPATHETIC GANGLION[1]

V. N. SHAMOFF, M.D.
PETROGRAD, RUSSIA

From the Laboratory of Surgical Research of the Harvard Medical School, Boston, Mass.

Received for publication November 4, 1915

In their studies on the autonomic control of the pituitary gland, Weed, Cushing and Jacobson (4) have shown that stimulation of the superior sympathetic ganglion on the neck of animals produced glycosuria even if all the nervous connections between the stimulated part and the abdominal viscera were completely severed. Comparing these experiments with those of direct excitation of the pituitary gland and with the "piqûre glycosurique" of Claude Bernard, both of which also may elicit glycosuria even when all impulses are shut off from the abdominal viscera, the authors came to the conclusion that the glycogenolytic effect of these excitations, acting upon liver and, possibly, on the adrenal glands with the resultant appearance of sugar in urine, could be explained only through the discharge into the blood stream of hormones of the pituitary gland.

From these investigations it may be concluded that the excitation of Bernard's point on the floor of the fourth ventricle, as well as the stimulation of the superior cervical ganglion, may send impulses which result in the secretory discharge of the pituitary body, through the cervical sympathetic system. This system, as has been histologically demonstrated by Dandy and Goetsch (2), sends terminal fibres to the pituitary body.

These interesting and important facts open a new method of attacking problems concerning the physiology of this organ which is placed in one of the most concealed and protected positions in the body. Up

[1] These experiments were performed during the summer of 1914, but under the difficult circumstances of the present war, the preparation of this paper for publication had to be delayed up to this time. Petrograd, March 23, 1915.

to the present time, the function of the pituitary gland was studied indirectly in two ways, viz., by excluding the organ through its extirpation, or by injecting its extracts into animals. Now, however, by exciting the superior cervical sympathetic ganglion, a structure comparatively easy to approach, we may produce the secretory discharge of the hypophysis directly, and proceed then to observations upon the effect of its hormones under more natural conditions.

The present study may be looked upon as a continuation of the work of Weed, Cushing, and Jacobson, and deals with the effect of the stimulation of the ganglion upon the urinary secretion, a matter which was only touched upon by these authors. Since Schäfer and Herring's (3) classical experiments, diuresis must share with the characteristic blood pressure response as one of the most typical phenomena produced by the injection of extracts of the posterior lobe.

In the technical part of my experiments on the flow of urine I applied Schäfer and Herring's methods. With the narcotized animal on its back, the urethra was exposed by a *sectio alta* and a glass catheter was introduced into the bladder through this incision. If the catheter was carefully inserted, no trouble was experienced in getting a regular flow of drops which were registered on the drum of the kymographion.

After insertion of the catheter, the regions of the two carotid arteries were exposed and in doing so, with least possible manipulations, the two vago-sympathetic trunks on both sides of the neck in their midcourse were divided. Then the tissues in the region of the left superior cervical ganglion were carefully dissected without actually exposing the ganglion itself, in order to avoid any traumatic excitation at this time. After inserting a cannula into the right carotid artery, the blood pressure, as well as the falling drops of urine and the respiration were all recorded.

After defining the normal blood pressure and the rate of urinary flow at this stage, the left cervical ganglion was then finally isolated and stimulated by a faradic current. The moment of the exposure and of excitation of the ganglion were noted on the tracing. Subsequently on disappearance of the primary reaction the excitation was repeated several times with increasing strengths of the current.

Almost all of these observations have been made on cats, for these animals, as shown by Schäfer and Herring, are the most suitable ones for demonstrating hypophyseal diuresis. Moreover, in cats the superior cervical ganglion is situated much lower in the neck than in other animals and is, therefore, more accessible and more quickly exposed;

then again, the stimulation of the ganglion can be made with greater exactitude. Only a few experiments of a preliminary character were carried out on dogs and rabbits.

The animals used for observations were in some cases anaesthetized by means of urethan, administered through a stomach tube in the dosage of 2 grams pro kilo. In other cases the animals were narcotized by the intratracheal insufflation of ether.

My first series of experiments consisted in stimulating the sympathetic ganglion subsequent to a division of the cervical nerves, as related. The results were fairly definite, as may be gathered from the following sufficiently typical protocol:

Experiment No. 7. June 11, 1914.

Cat, weight 3 kgm. Urethan, 6 grams.

Division of both vago-sympathetic trunks in the middle of the neck. Drops of urine issuing at intervals of 12 seconds each. The flow of urine soon after exposure of the superior sympathetic cervical ganglion increased in velocity to a drop every 2 or 3 seconds.

11.54 a.m. Faradic *stimulation* of the ganglion. Subsequent cessation of flow with a single drop only after an interval of 1 m. 12 seconds. This was followed by a distinct acceleration of drops of urine with about a 1 second interval (fig. 1).

The blood pressure which was at first about 104 mm., after the stimulation of the ganglion showed a short depressor phase to 88 mm. and subsequently rose to 148 mm.

The acceleration of the urine flow, which was often observed in cases such as the above, could not without reservations be ascribed to a pituitary discharge, for in experiments conducted in the above manner, some stimulation might have been transmitted by way of the spinal cord to the abdominal viscera.

Consequently in a subsequent series of experiments I proceeded still further, and not only divided the aforementioned nerves in the neck, but transsected the spinal cord as well, this being done at the fourth thoracic segment, i.e., above the level of emergence of the first splanchnics. When acutely performed, this operation commonly produces a decided decrease of blood pressure followed by a prolonged anuria, so that a preliminary aseptic surgical transsection of the spinal cord at T4 level was performed and not until full recovery was the observation made of stimulating the sympathetic ganglion.

With this preliminary transsection of the spinal cord, I have performed 15 experiments. Out of these, in eight cases the excitation of the sympathetic cervical ganglion resulted in decided diuresis, fully resembling that which follows an intravenous injection of posterior

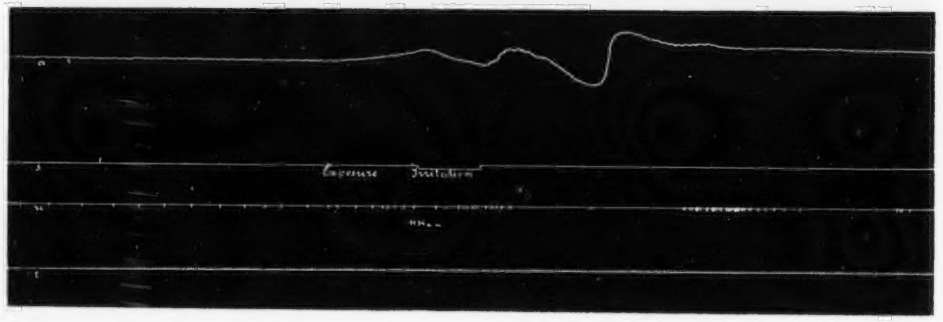

Fig. 1. Exp. N 7. *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *u*, urine flow; *t*, time in one-second intervals. Exposure and faradic irritation of the left superior sympathetic ganglion.

lobe extract as shown on Schäfer and Herring's tracings, and such as we ourselves have often observed.

In some cases immediately after the exposure of the superior sympathetic ganglion there occurred some acceleration in the flow, to be explained, I presume, by the fact that some mechanical irritation of the ganglion is inevitable during the manipulations. The acceleration of flow subsequent to faradization of the ganglion begins, as after an intravenous injection of hypophyseal extracts, after a short interval of time. This interval in some observations was quite insignificant, only a few seconds long, but in others it lasted from two to four minutes. If it occurred immediately, the reaction was usually pronounced, indicating either greater activity of the gland in the particular animal or a more successful discharge. Occasionally the drops of urine have increased ten-fold in their rapidity and after a long interval have returned again to the normal or even below it.

Repeated stimulations in the same animal with the same strength of current resulted in a reaction which, as a rule, was not so obvious, or even, as in the case of experiments No. 15 and No. 20, was, altogether, absent; but if on these subsequent stimulations the current was increased, it evoked an acceleration in the flow of urine, in some cases even on the second or third excitation. Doubtless the gland may become exhausted and in some instances after a repeated stimulation had ceased to produce any further reaction, an acceleration of flow could be produced after an intravenous injection of pituitary extract.

With regard to the influence of the ganglionic stimulation on the animal's blood pressure, we have almost invariably observed a primary fall and subsequent rise after each of the primary stimulations. These haemodynamic responses behaved much as the diuretic ones, for a repeated stimulation with a current of equal intensity produced a less obvious response or none at all (e.g., experiment No. 15). However, applying a current of a greater intensity at each subsequent stimulation, the modifications of blood pressure produced were as determinate as those occasioned by the first excitation (e.g., No. 22 and No. 29).

It should be emphasized that the acceleration of urine flow is independent of the elevation of blood pressure, for, as a matter of fact, it often takes place at the moment of the lowest depressor phase (experiments No. 15 and No. 29). It might be objected that the increased flow was due not to an augmentation of the secretory discharge of the kidneys, but that we are dealing with a mechanical modification of the bladder capacity from some reflex or other action. However, in the

experiments with a high transsection of the thoracic cord, there was a complete paralysis of the abdominal walls so that by no possibility could contractions of the abdominal musculature or other movements have influenced the urinary discharge.

In all these experiments the urine was tested for the presence of sugar before and after stimulating the ganglion. In a few cases sugar was found in small quantities before the stimulation, due to the glycosuria produced by and maintained after the spinal cord transsection. Still, even in these cases, after stimulation of the sympathetic ganglion the quantity of sugar was much augmented. In these experiments, in which before the stimulation the urine was sugar free, sugar usually appeared after the stimulation, just as was observed in the experiments of Weed, Cushing and Jacobson.

The following are some of the typical protocols of the second series of experiments.

Experiment No. 15. Cat, weight 2700 grams.

Transsection under ether of the spinal cord at 4th thoracic segment, 4 days previous to the beginning of the experiment. All aseptic precautions observed. No complications.

July 10, 1914. Division of both vago-sympathetic trunks under urethan anaesthesia. Catheter introduced into the bladder and cannula into the right carotid artery. Dissection of tissues in the region of the left superior sympathetic ganglion.

11.25 a.m. Kymographion record started. Urine flow in drops with intermissions varying from 18 to 25 to 30 seconds; blood pressure 98 mm.; a small trace of sugar in urine.

During the exposure of the ganglion and coincident with the period stimulating it, a few drops of urine issued with intermissions of 9 to 2 seconds.

11.30 a.m. Faradic *stimulation* of the ganglion for 20 seconds. This was almost immediately followed by an acceleration of urine flow, with drop intermissions reduced to only 2, 1, and even ½ second each (fig. 2).

Blood pressure previously at 98 fell to 84 and then rose to 108 mm. The period of accelerated urinary flow coincided with the depressor phase.

11.50 a.m. Intermissions between the drops of urine now average 35 seconds. *Second stimulation* of the ganglion with the same strength of stimulus without appreciable effect on urine or blood pressure except for a slight depressor response.

12.17 p.m. Third stimulation with the same strength of current, but for a longer period. No appreciable effect except for a slight depressor response. Urine shows a heavy sugar reduction.

12.25 p.m. Intravenous injection of 1 cc. of Armour's "pituitary liquid."[2] No effect on urine; slight modification of blood pressure from 88 to 76 to 94 mm.

[2] This preparation, as we can conclude from a series of our observations, does not usually lead to the polyuria which is so characteristic of other hypophyseal preparations.

Experiment No. 20. Cat, weight 3700 grams.

Under ether; transsection of the spinal cord at T. 4, six days previously. All aseptic precautions taken.

July 22, 1914. Urethan, 7.4 grams. Both vago-sympathetic trunks sectioned in the middle of the neck; a catheter inserted into the bladder and a cannula into the right carotid artery. Dissection of tissues in the region of the superior sympathetic ganglion.

10.30 a.m. Kymographion registration started. Drops of urine issuing with an average interval of 35 to 45 sec. Blood pressure 98 mm. No sugar in urine.

10.51 a.m. Exposure and *stimulation* of the ganglion by faradization. An almost immediate acceleration of urine flow with shortened interval to 11 or 12 seconds (fig. 3). Blood pressure rises without primary fall from 98 to 108 mm. Urine shows a marked reduction of sugar.

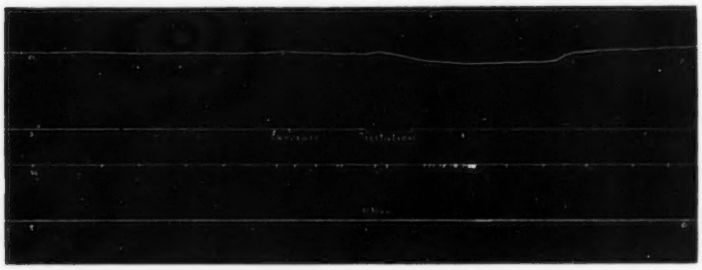

Fig. 2. Exp. N 15. *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *u*, urine flow; *t*, time in one-second intervals.

11.35 a.m. The intermissions between drops average about 90 seconds. *Second stimulation* with an identical current. Cessation of urine flow. Slight pressor response from 96 to 108 mm.

12.24 p.m. No urine appearing. *Third stimulation* with the same strength of stimulus. No urine flows. Slight depressor response from 92 to 82 to 92 mm.

12.36 p.m. Intravenous injection of 0.9 cc. of "hypophysin." Three minutes later drops of urine began to issue with intermissions of 60 to 70 seconds. Modifications of blood pressure from 92 to 74 to 116 mm.

Experiment No. 25. Cat, weight 1700 grams.

Transsection under ether of the spinal cord at T. 4, three days previously. All aseptic precautions taken.

August 2, 1914. Anaesthesia by interrupted intratracheal insufflations of ether. Both vago-sympathetic trunks sectioned; catheter and canula as before; dissection of the superior sympathetic ganglion.

10.45 a.m. Kymographion tracing started. During the 6 minutes previous to stimulating the ganglion, there was complete cessation of urine flow. Blood pressure at 90 mm.

Fig. 3. Exp. N 20. *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *u*, urine flow; *t*, time in one-second intervals.

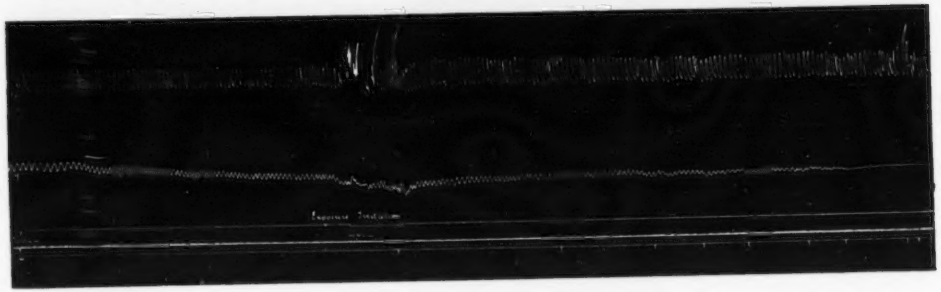

Fig. 4. Exp. N 25. First stimulation of respiration; *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *t*, time in one-second intervals; *u*, urine flow. No urine was being secreted before the irritation.

10.51 a.m. *Stimulation* of the ganglion by faradization. At the last moment of the excitation corresponding with the period of lowest blood pressure, appeared the first drop of urine; the second drop came out two minutes later, the third in one minute, and then drops issued in 30 to 15 to 8 second intervals. The blood pressure showed merely a short depressor phase from 90 to 64 to 90 mm. (fig. 4).

11.38 a.m. The intermissions between drops now average about 50 to 85 seconds. *Second stimulation* of longer duration applied to the ganglion followed by a primary suppression of urine for 3 minutes succeeded by an acceleration with issuance of drops every 35 to 20 to 10 seconds. Blood pressure reaction from 84 to 66 to 80 mm.

12 noon. Average interval between drops equals 47 seconds.

Third stimulation by a stronger current. Urine flow checked for 90 seconds followed by an acceleration with intervals of 20 to 8 to 5 seconds. Modification of blood pressure from 80 to 62 to 86 mm. Heavy sugar reaction in urine towards the end of the experiment.

Experiment No. 29. Cat, weight 2000 grams.

Spinal cord transsection at T. 4, three days previously. All aseptic precautions taken. No complications.

August 9, 1914. Intratracheal anaesthesia of ether without interruptions and usual preparation with division of both vago-sympathetic trunks.

3.04 p.m. Kymographion registration started. Drops of urine issuing every 37 to 47 seconds; blood pressure 116 mm.; urine negative for sugar.

3.18 p.m. *First stimulation* (coil at 1.5 cm.) followed by acceleration of urine, the intervals being reduced to 28 and 25 seconds. Blood pressure reaction from 116 to 82 to 144 mm.

3.34 p.m. Intermissions in urine drops lengthened to 30 to 42 seconds. *Second stimulation* with a stronger current (coil at 2 cm.) followed by increase of drops issuing at every 13 to 10 seconds. Characteristic blood pressure response from 114 to 48 to 140 mm. (fig. 5).

4.17 p.m. Drops of urine have slowed to every 40 to 50 seconds. *Third stimulation* with a stronger current (coil at 2.5 cm.) followed again by an acceleration of urine flow; drops issuing every 30 to 23 seconds. Typical blood pressure response from 104 to 80 to 134 mm. A large amount of sugar present in the urine.

Equally positive results were obtained in 8 of the 15 experiments in which a preliminary spinal transsection was performed. In 5 of the remaining 7, there was a certain acceleration of the flow, though it was too insignificant to speak positively regarding it; whereas in the remaining two experiments there was no acceleration of the flow whatever.

These negative results are possibly no more numerous than might be expected, for Schäfer and Herring have pointed out that, in spite of all precautions, the "operative procedure and the effect of the anaesthetic combined, frequently produced suppression of urine flow, so that in some experiments this part of the record is negative throughout."

In our experiments, the operative traumatization was necessarily far

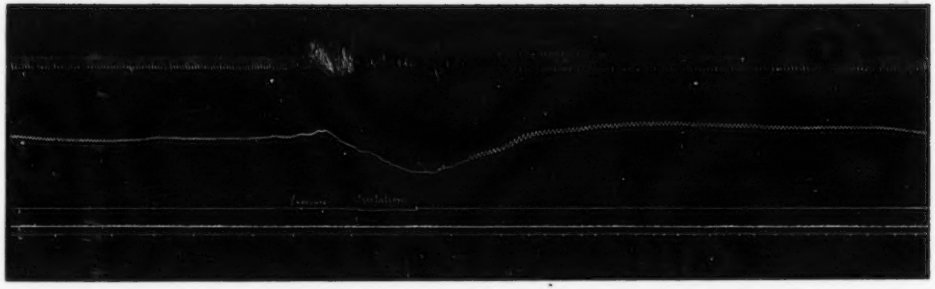

Fig. 5. Exp. N 29. *r*, respiration; *a*, arterial blood pressure; *s*, signal and abscissa of blood pressure; *t*, time in one-second intervals; *u*, urine flow. At 3.34 p.m. the second stimulation of the ganglion.

greater than that inflicted by Schäfer and Herring, whose total operative procedure consisted merely in the insertion of the cannula into the carotid and of a catheter into the bladder. Furthermore, in place of injecting intravenously a comparatively large quantity of hypophyseal extract of other animals, we attempted to produce a more or less natural secretory discharge of the pituitary gland of the experimental animal itself. Under these conditions we might expect a far less pronounced reaction, as well as considerable fluctuations of it, depending on the state of the organ in each separate animal at the time of the experiment. In all likelihood, the secretory power of the gland is influenced by the state of the organism, and the preliminary spinal cord transsection may have considerably reduced our chances of producing a normal discharge from the pituitary gland. Thus the unfavorable factors, to which Schäfer and Herring refer, must have been considerably increased by the major procedure necessary for our experiments.

In addition to the above-mentioned factors, there is the further consideration pointed out by Schäfer and Herring, namely, the presence of a substance in hypophyseal extracts which acts as a vasoconstrictor and another as a vasodilatator; consequently in some cases "the vasoconstrictor may overpower the vasodilatator substance in acting upon the renal vessels, and this would naturally lead to a diminished secretion of urine as long as the constriction of renal vessels lasts, and for that period, at least, the extract would be antidiuretic rather than diuretic." Very likely the discharge of different substances of the pituitary gland is occasioned by strictly determinate different factors in the organism, and possibly they are modified by variable nervous impulses passing to the gland. A hint as to this can perhaps be found in the fact that in some of our experiments, after a repeated irritation, instead of an acceleration of urine flow a temporary retardation occurred. We of course cannot exclude the possibility of stimulation of different parts of the gland through irritation of one or another part of the cervical ganglion. From this point of view the failure to obtain diuresis in a number of our experiments can possibly be explained.

As a matter of fact, the negative records in the series of experiments do not serve to offset the positive responses. But can even the positive records be looked upon as definite? And can the diuresis seen by us be really explained as the effect of hypophyseal secretion, discharged by the stimulation of the superior sympathetic ganglion? This deserves an affirmative answer, inasmuch as our experiments have involved a

total exclusion of all nervous impulses from the stimulated ganglion down to the kidneys and hence we can conceive of only one way of influencing their activity and that is through the blood by the diuretic hormone of the posterior lobe. The gland that produces the substance, in spite of the division of both vago-sympathetic nerves and the transsection of the spinal cord at the fourth thoracic segment, remains connected by a nervous pathway with the superior sympathetic cervical ganglion. As to its being actually the pituitary body is demonstrated by all reactions typical for its secretion, for after the stimulation of the sympathetic cervical ganglion (1) there usually occurs a characteristic haemodynamic response; (2) as was observed by Weed, Cushing and Jacobson, as well as by myself, glycosuria is provoked; and finally, (3) a polyuria, resembling that produced by intravenous injection of pituitary body extracts, frequently occurs.[3]

Thus, the positive records of my experiments fully coincide with the data of my predecessors in this laboratory but indicate further that stimulation of the superior cervical sympathetic ganglion leads to a discharge of hypophysial secretion which produces diuresis, as well as glycosuria.

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[3] Lately Camus and Roussy (1) have published some experiments to prove that the "polyurie dîte hypophysaire" is due to a lesion "de la region interpédonculaire de la base du cerveau" rather than to that of the pituitary body itself. Similarly Aschner and others have chosen to explain the so-called hypophyseal glycosurias, adiposity, drowsiness and so on to injury of predicated centres in the floor of the third ventricle. The main results of the experiments of Camus and Roussy are that polyuria is produced not only in cases of experimental lesion of the pituitary body itself, but also in lesions of the basis cerebri situated immediately behind it, and that in the latter case the lesion was accompanied by polyuria even after a preliminary extirpation of the pituitary gland. On the contrary, in cases where the extirpation of the hypophysis was performed with special precautions, so as not to traumatize the cerebral base, it produced in animals no polyuria whatever. These observations deserve consideration and repetition, but the prevalent view of most investigators is that these phenomena are of hypophyseal rather than of encephalic origin.

THE ORIGIN OF THE CARDIAC IMPULSE IN THE TURTLE'S HEART

WALTER J. MEEK AND J. A. E. EYSTER

From the Physiological Laboratory of the University of Wisconsin

Received for publication, November 5, 1915

The cardiac physiology of the cold-blooded heart has been found applicable to the mammalian heart in practically all of its details. This fact has lead certain investigators to doubt the propriety of assigning the seat of impulse formation to the sino-auricular node in the mammalian heart unless it could be shown that the homologous structure of the cold-blooded forms was also a motor center. Erlanger (1) in particular has voiced this view.

Since the work of McWilliam, Gaskell and Engelmann it has been accepted with but few exceptions (2) that the heart beat in the cold-blooded animals arises in the sinus. This we have ourselves confirmed by the newer electrical methods (3). So far as we are aware, however, no attempt has ever been made to discover whether the sinus acted as a unit or whether certain of its parts were preeminent in automaticity. Certainly this point was not touched in our previous publication.

With the bulk of the recent work emphasizing the function of the sino-auricular node as pacemaker in the mammalian heart, two important questions from the comparative physiological standpoint arise: first, what part of the primitive heart does the sino-auricular node represent; and second, does the beat originate in this homologous structure in the lower vertebrate forms.

As to the first of these questions there are now anatomical investigations extant which we believe furnish a correct answer. His (4), as is well known, found by embryological methods that the portion of the mammalian heart designated by him as sinus reuniens represented the sinus of the lower forms. Although he found a complicated fusion of sinus and auricle he nevertheless felt able to locate the boundaries of the former. The sulcus terminalis on the exterior and the taenia terminalis on the interior marked the line of separation between the sinus reuniens and the pectinate musculature of the auricle.

The sino-auricular node, being found in the sulcus terminalis, would then, according to the conceptions of His be a structure occurring at the sino-auricular junction. But even with this knowledge one could not be quite sure whether the node really belonged to the sinus side of the junction. It might of course be an auricular structure shifted to this position during the period of cardiac development. The exact location of the sino-auricular node in the mammalian heart has been determined in a very important piece of work done by Oppenheimer and Oppenheimer (5). These investigators examined with great care the hearts of two foetuses and one child. In these hearts they found the venous valves, structures which in lower forms separate the sinus from the auricular canal. That Oppenheimer and Oppenheimer actually found the venous valves can scarcely be doubted since they were identified by shape, structure and location. The identification of the valves of course gave a land mark for the exact location of the sino-auricular node. The node was easily discernible at the base of the venous valves in the region covered with a thick endocardium; in other words in the tissue which corresponds to the sinus venosus of cold blooded vertebrates.

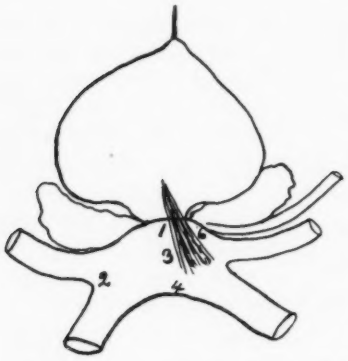

Fig. 1. Ventral outline of turtle's heart with the ventricle pulled up and forward to expose the sinus. Figures indicate the position of the electrodes on the sinus.

The exact location of the sino-auricular node seems then to be on the sinus side of the sino-auricular junction. This region of the lower forms has been examined histologically by Keith and Flack (6) and by Keith and Mackenzie (7). They report at the sino-auricular (sino-canalar) junction, a network of fine, primitive palely staining fibers evidently the homologue of the sino-auricular node in the mammalian heart. In the tortoise Keith and Mackenzie describe this nodal tissue as being in intimate connection with the right auricle although its chief center is at the termination of the pulmonary vein. This vestibule of the pulmonary vein they have shown to be of sinus origin. The nodal tissue really makes a circle around the sino-auricular junction with its chief mass on the left side of this ring.

The second question, that in regard to what part of the cold-blooded sinus functions as pace maker, we have taken up in the present paper. Our method has been to determine the point of initial negativity in the sinus by means of the string galvanometer. Large mud turtles, Chelydra serpentina, were used. The animals weighed about 30 pounds and the sinus was often found to be as much as 4 cm. wide. We have now studied eight of these hearts and the results have been consistent enough to justify reporting the series.

After pithing, the carapace was removed. The heart was exposed and the ventricle raised and tilted forward thus completely exposing the sinus region. Figure 1 gives an outline of the parts with numbers showing the position of the electrodes. These were non-polarizable of the zinc-zinc sulphate type. From the end of the glass tubes forming the electrodes, hung woolen threads soaked in salt solution which could be applied to any part of the heart. They were watched throughout the experiment to insure their remaining in the same position. The contraction of the sinus was identified either by referring to the auricular contraction which was recorded by air transmission in front of the photographic slit, or by means of a similarly recorded signal from the observer. In all cases after records were taken from the undisturbed heart the second Stannius ligature was laid and the observations repeated. This series has been considered the most important since in some cases the T wave of the ventricle had obscured the sinus deflection. A sen-

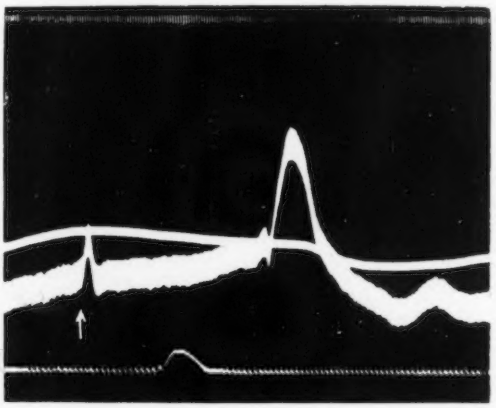

Fig. 2. A comparison of the sino-auricular ring (1) with the right edge of the sinus (2). The right hand electrode is on point 1, hence the up stroke of the sinus wave indicates primary negativity of the sino-auricular ring. The ventricle has been tied off and is quiescent. The second wave is auricular. An arrow marks the sinus wave. The other lines are the signals, the upper from the auricle, the lower from the observer.

sitive string was used, 1 millivolt deflecting the string through 29 cm. at the distance of the photographic slit.

The positions at which the electrodes were placed may be seen by referring to figure 1. Point 1 was over the left ventral quarter of the sino-auricular ring; 2 was at the extreme right of the ventral wall of the sinus; 3 was slightly to the left of the middle of the ventral sinus wall; 4 was at the posterior edge of the ventral sinus; and 6 was over the extreme left edge of the sino-auricular ring. Our results are briefly as follows:

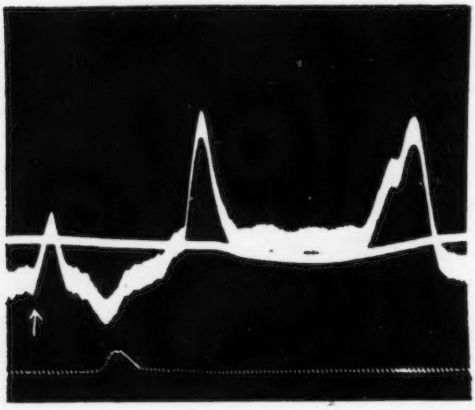

Fig. 3. Sino-auricular ring (1) compared with the posterior edge of the sinus (4). Up stroke of the sinus wave indicates primary activity of the sino-auricular ring. The sinus wave is here followed by the auricular and ventricular deflection.

Point 1 was negative before point 2 in 8 of 8 experiments.

Point 1 was negative before point 4 in 7 of 8 experiments.

Point 1 was negative before point 6 in 6 of 7 experiments.

Point 1 was negative before point 3 in 4 of 5 experiments.

Point 1 was thus found to precede regularly all other parts of the sinus. In other words it would seem that in the eight turtles examined by us the cardiac impulse was in all cases being initiated in some part of the sino-auricular ring. It will be noted that point 1 was preceded by points 6, 4 and 3 in one case each. These exceptions all arose in one experiment in which it was found that the beat was arising at the extreme left edge of the sino-auricular ring under electrode 6. As the excitation spread points 3 and 4 became involved before 1, but as might be expected 1 still preceded 2. That the region under electrode 6 should at times be pacemaker is not surprising since it was here in the tortoise that Keith and Mackenzie (7) found the largest masses of specialized tissue. They noted that there was a tendency even in the cold-blooded forms for this tissue to shift toward the right and thus come in still more intimate rela-

tion to the right auricle. Such a shifting might well explain the fact that point 1 usually precedes point 6 in activity in the forms studied. Even if the pacemaker is found at times in the region of point 6 it in no way modifies our conclusion that the beat starts in the sino-auricular ring presumably in the left portion. Point 3 was compared with points 2 and 4 and found regularly to precede them in activity. These leads served as a control and further proof that excitation was spreading from the sino-auricular junction.

Our results justify the conclusion that even in the cold-blooded hearts the cardiac impulse originates in a definitely localized portion. This motor center is in the sino-auricular ring where Keith and Mackenzie have described a specialized muscle tissue resembling in most of its characteristics that found in the nodes of the mammalian heart. All the data at present available, both anatomical and physiological support the generalization that in all vertebrate hearts the seat of cardiac impulse formation is located at the sino-auricular junction in masses of the so-called specialized tissue.

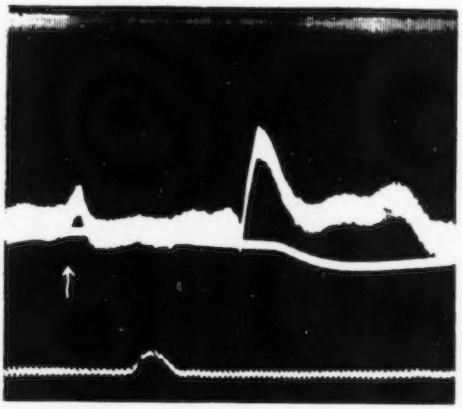

Fig. 4. A comparison of the ventral part of the sino-auricular ring with its extreme left margin. Up stroke indicates primary negativity of the ventral portion rather than the left edge of the ring. The ventricle has been tied and its deflection does not appear.

SUMMARY

Electrocardiographic examination of the turtle's heart shows that the initial point of negativity during each cardiac cycle is in the sino-auricular junction. It is in this region that specialized tissue is found, both in the cold-blooded and mammalian hearts. All vertebrate forms then seem to agree in having the pacemaker of the heart located at the sino-auricular junction in masses or nodes of specialized tissue.

Since writing the above our attention has been called to an important paper by Laurens on the "Connecting Systems of the Reptile Heart" (*Anatomical Record*, 1915, ix, p. 427). Laurens finds that in the tortoise heart the best connection between the sino-auricular ring and the right auricle is at the position of the right venous valve. On the left side the fibers are divided between auricle and septum. Our experiments, which to our surprise showed the pacemaker to be somewhere to the right of the left venous valve, are therefore in harmony with Lauren's anatomical findings. It is probable that if different parts of the sino-auricular ring were compared the pacemaker would be found near the right venous valve. Our work of course has not taken up this point but has been content with showing that the seat of impulse formation was in the sino-auricular ring rather than in the body of the sinus.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE
NO. 267

IONIC ANTAGONISM IN SENSORY STIMULATION

W. J. CROZIER

Received for publication November 8, 1915

This note is concerned with a demonstration of the fact that laws of salt antagonism are applicable to certain phenomena of sensory stimulation by electrolytes. Such an antagonism may readily be demonstrated for the salts of sea water when marine animals (Holothurians, Ptychodera) are the subject of experiment. According to Parker and Metcalf (1) the reactions of the earthworm Eisenia foetida to mixtures of NaCl and $CaCl_2$ are not such as to indicate an antagonistic effect of these salts in their action on the chemoreceptors of the worm; but their study dealt with only a few concentrations. The data with reference to human taste are in too chaotic a state and too complex to admit of analysis at present.

Material used in the present experiments comprised some 54 frogs, Rana pipiens, ranging in length (snout to anus) from 60 to 87 mm. The experiments were all made upon autumn frogs, during October and November, 1914. The method followed was, to all intents and purposes, that of Cole (2). The frogs were prepared by pithing and hanging upon a platinum hook passed through the lower jaw; care was taken that the frogs were in good condition and that the feet were not abraided. About one-half hour elapsed between pithing and the first stimulation trials, during which the skin was kept moist with tap water.

In measuring the reaction time a frog's foot was immersed in a given solution up to the proximal end of the digits. It is worth while mentioning that the area of the foot exposed to the solution does not have to be constant, since only very slight and inconstant variations in reaction time are found when the responses are compared for immersion of (a) the whole foot and (b) one-half the length of the digits. The salt studied were NaCl, KCl, and $CaCl_2$. The trials on individual frogs were so rotated among solutions of these salts that fifteen reaction times

TABLE I

Reaction time of frog's foot, seconds. R. and L. signify right and left feet respectively

October 23, 1914 Frog 47 (62 mm.). (19°6)			October 24, 1914. Frog 49 (66 mm.). (20°0)		
SOLUTION	R	L	SOLUTION	R	L
NaCl, 2M	11.9	6.2	$CaCl_2$, 0.1M	∞ *	∞
	6.2	10.5		∞	∞
	7.1				∞
$CaCl_2$, 1M	8.0	6.7	NaCl, 1.5M	4.3	8.3
	12.9	11.4		6.3	12.0
		10.6		13.0	
NaCl, 2M, 50 cc. {	10.1	11.2	NaCl, 0.75M	58.9	45.0
$CaCl_2$, 1M, 50 cc. {	14.4	16.0		34.5	55.1
	14.8				28.7
NaCl, 0.5M	44.2	65.6	NaCl, 2M, 25 cc. }	10.3	10.6
	42.5	29.4	$CaCl_2$, 1M, 75 cc. {	13.9	15.2
		25.7		9.7	

* N.B. ∞ = no reaction (i.e., infinite reaction time).

TABLE II

Reaction time of frog's foot, seconds. Averages of 15 measurements

SALT	NaCl	KCl	$CaCl_2$
Conc.			
2.0M	11.5		
1.5M	8.31		
1.0	26.8	4.36	10.4
0.75	44.4		14.3
0.50	40.8	5.36	37.3
0.25	∞	10.5	149.
0.10		25.6	∞

TABLE III

Reaction time to mixtures of NaCl *and* $CaCl_2$.
NaCl, 2M and $CaCl_2$, 1M.
*x*cc.A + *y*cc.B

90A + 10B	75A + 25B	50A + 50B	25A + 75B	10A + 90B
13.4	14.3	13.3	11.8	11.3

were measured for each concentration, using three frogs to give five reactions each, but the order in which the successive salts were tried was varied from frog to frog in such a way as to rule out any after effect of a particular solution. By way of illustration the records of frogs 47 and 49 are given in detail (Table I). The temperature during these experiments varied from 18° to 21°. After each test the foot concerned was washed in tap water.

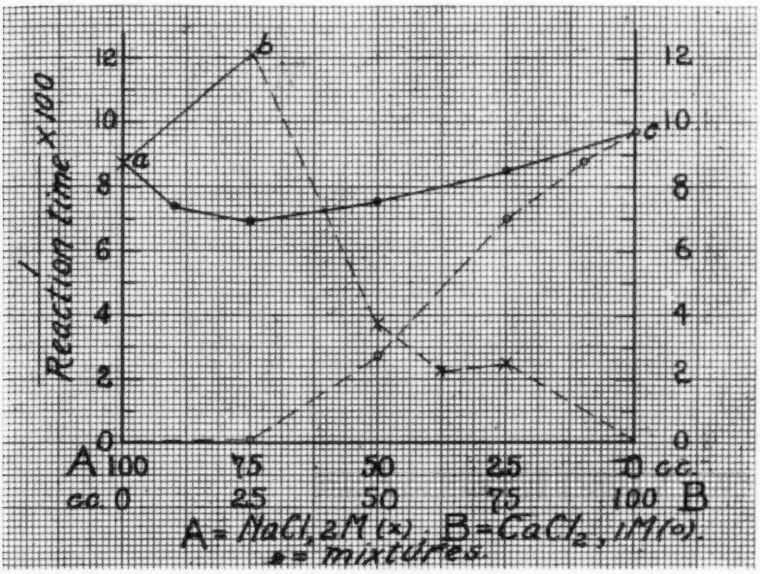

Fig. 1. Reaction time of the frog's foot to mixtures of NaCl and $CaCl_2$. For explanation, see text.

Data for solutions of the single salts, NaCl, KCl, and $CaCl_2$, are summarized in Table II.

If the results of Cole(2) in a similar study be compared with the values of Table II, it will be noted that his frogs were rather less reactive and more variable than mine, and further that there is not a very good concordance in the individual averages. This is mainly to be accounted for, I believe, by the fact that Cole's work dealt with spring frogs; size variations are also liable to be important.

It was found at the beginning of these experiments that, while 0.5M NaCl gave a weak but measurable stimulus, sea water (0.6M) was totally inefficient in this respect. Mixtures of NaCl and $CaCl_2$ were then studied, with the resuls given in Table III, and shown graphically in figure 1. In figures 1 and 2 the *reciprocals* of the reaction times have been plotted; the method of representation is a variation of that suggested by Osterhout (3), with the addition of the dilution curves for the individual components of the mixtures; the variation consists in

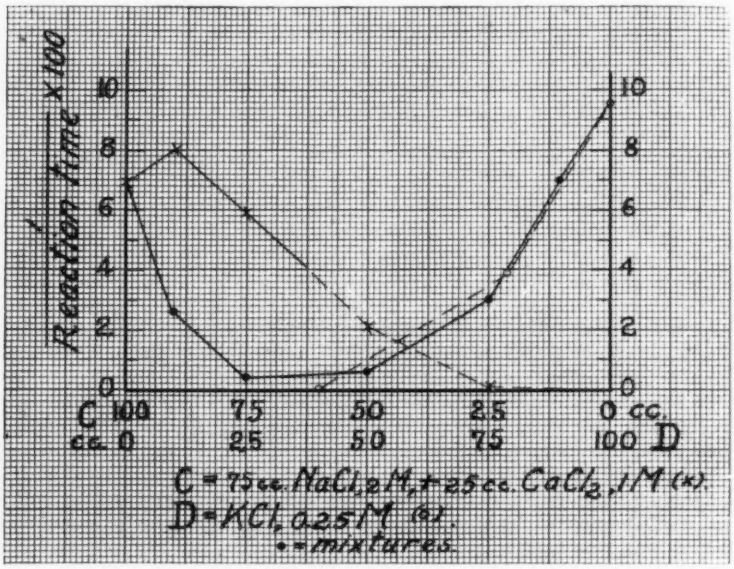

Fig. 2. (See text.)

plotting, not molecular percentages, but volume percentage compositions, as abscissas. Since reciprocals of reaction times (i.e., "stimulating powers" of the solutions) are plotted, antagonism of one component of the mixture by another would be shown by the curve for the mixtures lying below the theoretical "curve of no mutual action," and by its being convex toward the axis of abscissas; but the occurrence of a convexity in this sense does not necessarily indicate an antagonistic effect, because the nature of the base line for measuring antagonism in Osterhout's method cannot in general be determined with accuracy.

The "base line" referred to is given by Osterhout (3), for the case when *A C* and *B D* are equal, as the straight line connecting the ends of the ordinates which represent the activity of the solutions of each of the components of the mixture. As a matter of fact, such a base line, giving the additive effect of mixtures between solutions *A* and *B*, were there no mutual interaction of the substances, could only result if the *dilution vs. effect* curves for the individual components were of exactly identical forms;[1] a base line of the required significance might then be calculated. This point is equally applicable when the percentage composition of the mixtures is expressed in terms of the molecules present.

TABLE IV

Reaction time to dilutions of NaCl + $CaCl_2$
75 cc. NaCl, 2M + 25 cc. $CaCl_2$, 1M and Water.

*x*cc.C + *y*cc.W				
90C + 10W	75C + 25W	50C + 50W	25C + 75W	10C + 90W
12.5	16.9	49.7	99.5	∞

TABLE V

Reaction time to mixtures of NaCl, $CaCl_2$, and KCl.
75 cc. NaCl, 2M + 25 cc. $CaCl_2$, 1M and KCl, 0.25 M.

*x*cc.C + *y*cc.D				
90C + 10D	75C + 25D	50C + 50D	25C + 75D	10C + 90D
38.4	250	166	33.5	14.3

In the case recorded here the activity of the mixed solutions was higher than that of the corresponding concentrations of the single substances, except for the mixtures having 75 per cent and 90 per cent NaCl; the curve has a minimum at 75 cc. NaCl + 25 cc. $CaCl_2$. It is important to note that this effect of the $CaCl_2$ is apparent in a mixture of which the $CaCl_2$ component has *by itself* no stimulating action—hence the "curve of additive effect" would for this case be that portion (*a*, *b*) of the NaCl curve which is drawn as a full lines (cf. also figure 2).

[1] It is hardly necessary to remark that such a condition is in reality never found with solutions of different salts.

This mixture of NaCl and $CaCl_2$ was used as one component of mixtures containing KCl. The dilution curves for these components and for the three-salt combinations are shown in figure 2, plotted from the data of Tables IV and V. In the mixture containing 75 cc. (NaCl + $CaCl_2$) and 25 cc. KCl there is apparent a minimum of stimulation; the solution contains the three salts in the ratio Na: K: Ca :: 12: 1:8. The Na : K ratio (12 : 1) at this point is not far from that found by Loeb (4) for antagonism in various concentrations of Na and K salts (17: 1). This mixture stimulated to a measurable extent, while sea water did not; it is uncertain whether the lower total concentration[2] of the sea water or the presence of additional salts determined its nonstimulation.

From the fact that antagonism is evident in the stimulation of the frog's foot by salts, it seems legitimate to conclude that in normal stimulation the essential step includes the penetration of the surface layer of the receptor by the stimulant.

POSTSCRIPT

I have recently (December 4, 1915) received a copy of a paper by Professor Osterhout in which an attempt is made to correct the determination of the "curve of additive effect." In this paper, (Bot. Gaz., vol. 60, 1915, pp. 228–234) the method of measuring antagonism originally proposed as generally valid, is apparently restricted to *toxic* effects. It seems only fair to state that the present paper was written without knowledge of Professor Osterhout's later communication. As a matter of fact, the existence of the complication above referred to, and the necessity for detailed consideration of the dilution curves, were first pointed out by myself in conversation with Professor Osterhout in October, 1914.

Agar's Island, Bermuda,
December 4, 1915

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[2] There is no a priori justification for any expectation that the salt ratios at a point of maximum antagonism will be identical for a range of (total) concentrations.

CONTRIBUTION TO THE PHYSIOLOGY OF THE STOMACH

XXXI. The Effect of Experimental Partial Stenosis of the Pylorus on the Motility of the Empty Stomach

OSCAR J. ELSESSER

From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 15, 1915

It is well known that partial stenosis of the pylorus in man leads to altered motility, tonicity (and secretion) of the filled stomach to a greater or less degree. Some attempts have been made to reproduce this condition in experimental animals in order to analyze their physiological significance. Pernice (1) in 1890 produced a partial stenosis of the pylorus in dogs by tying a sterile string under some tension around that sphincter. He merely noted the constitutional effects of his procedure on the animals. Roger and Garnier (2) in 1906 completely occluded the pylorus of three dogs by means of a rubber band. The dogs died before the fifth day and presented stomachs greatly distended with gas and serous exudate. Tuffier and Bonamy (3) in 1898, also working on dogs, endeavored to produce varying degrees of stenosis, using silk ligatures passed around the pylorus. Complete occlusion caused the death of one animal in seven days, which at autopsy showed merely a slightly dilated stomach. Partial occlusion produced practically no effects, the stomach evidently overcoming the hindrance. With partial chronic stenosis, it was found after twenty days, that the stomach filled the abdomen, "driving back all the viscera." The contents were liquid and of increased acidity. These authors venture the opinion that other results than these mentioned due to partial stenosis cannot be produced to any extent in less than two months duration. More recently Bolton (4) constricted the pylorus in cats, the results being varying degrees of motor insufficiency of the stomach, i.e., delay in emptying its contents into the duodenum. Hamburger and Friedman (5, 6) in their work on the genesis of gastric ulcer in dogs also constricted the phylorus by drawing a ligature about it. Depending on the degree of constriction they noted (a) little or no change in stom-

ach motility, acidity or size; (b) motor insufficiency, hyperacidity and hypertrophy and dilatation of the stomach, and (c) with complete occlusion death of the animal in from 48 to 120 hours.

Sick (7) made actual tracings (balloon method) of the contractions of the human stomach in a case of partial stenosis of the pylorus. Leonard (8), Case (9), Cole (10) and others have made extensive use of the X-ray in studying the motility of the filled stomach both under normal and pathological conditions. Hertz (11), Russell (12) and Maylard (13, 14) mention hypermotility, distension, etc., of the stomach in case of pyloric obstruction. Backman (15) presents an instance of pyloric stenosis with very large dilatation of the stomach but without motor insufficiency. Jonas (16) states that antiperistalsis of the stomach was almost always associated with pyloric stenosis.

These observations refer to the digestive motility of the stomach, that is, to the filled or partially filled stomach. Are the changes in gastric motility and tonicity in evidence only when food is present in the stomach and a part therefore of the normal mechanism by which the stomach tends to empty itself, or is there a fundamental change in the mechanism, i.e., chemical, sensory, neuromuscular, which persists even when the stomach is empty? In other words, does partial pyloric stenosis bring about changes in the motility and tonicity of the stomach which are present whether the latter is empty or not? The following experiments were carried on at the suggestion of Professor Carlson, with the view of answering this question.

EXPERIMENTAL PROCEDURE

Two young healthy female dogs, weighing between 5 and 8 kgm. were chosen. Both animals proved very tractable to the experimental technique and readily accustomed themselves to it. This consisted in swallowing a rubber condum attached to a firm rubber tube, which in turn was connected to a chloroform manometer, the latter transmitting the stomach contractions to a writing point on a slowly revolving drum. Tracings per os are to be preferred in this work to those taken per gastric fistula for the obvious reason that in the latter case we should have to deal with adhesions which might modify the stomach motility. The dogs, while the records were being taken, would lie quietly on their side, without any restraint of any sort, frequently sleeping complacently for periods of several hours—the work being carried on in a room as free from disturbing factors as possible. A series of normal tracings

were first recorded from each dog, comprising those taken immediately after feeding, and after twenty-four hour and forty-eight hour periods of starvation, whereupon the pylorus was partially stenosed according to a technique suggested by Dr. Carlson. The abdomen was opened

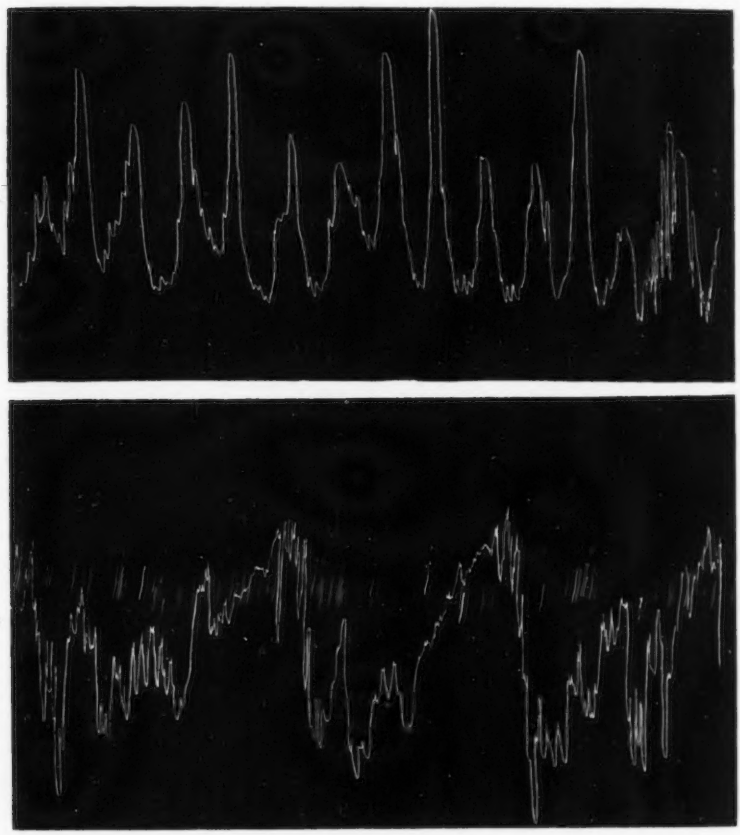

FIG. 1. Tracings showing contraction of the stomach of Dog A. Dog normal, the tracing, taken forty-eight hours after feeding, represents the height of a period of hunger contractions. B, same dog as in tracing A, forty-two hours after feeding, 30 days after production of partial stenosis of the pylorus, showing typical hypertonus and tetany periods of the empty stomach. Chloroform. Manometer. Time: 15 minutes.

under general anesthesia and the pyloric end of the stomach located. On being drawn gently through the incision, an area of serosa extending across the pyloric sphincter about $\frac{3}{4}$ cm. by $1\frac{1}{2}$ cm. was scarified, care being taken to avoid injury to the larger blood vessels of that region. Two rows of stitches running parallel to the long axis of the bowel were made, the second folding in the first, thus bringing the two scarified surfaces together and at the same time partially occluding the pyloric lumen. The method has these advantages, in that some pyloric obstruction is sure to be produced which will be firmly maintained by adhesions forming between the two raw surfaces. Furthermore, complete occlusion and loss of the animal is avoided, a termination frequently attendant upon placing ligatures around the pylorus. After the animal had recovered sufficiently from the effects of the operation—a matter of several days—another series of records were made duplicating the normal ones both as to conditions and periods of time. On one dog which we may designate as dog A, a second operation similar to the first was performed and a second series of tracings recorded. At all times the dogs were fed standard meals consisting of 170 grams and 180 grams respectively, of boiled, ground steak.

Dog A remained very well and active and was killed 35 days after the first operation and 14 days after the second. At autopsy the pylorus presented a lumen constricted by a hard tough mass of granulation tissue, the seat of the scarification and stitching. The stomach was not distended, but did show some hypertrophy. The gastric mucosa too lay in deep folds. There were no stomach contents, the animal having been given $\frac{1}{20}$ gr. of apomorphine previous to killing for another purpose.

Dog B became somewhat emaciated after stenosis, evinced a hypersensitivity of the gastric mucosa by frequently vomiting up the balloon while records were being made, and was found dead 7 days after the operation. Death could not have been due to complete pyloric obstruction as the animal continued to pass faeces after the operation. At autopsy the pylorus was constricted so that the stomach contents could be forced through it only with difficulty. The stomach itself was considerably dilated and contained much serous exudate, and meat from a previous meal.

Both dogs gave evidence of delayed emptying of the stomach after stenosis in that food remnants were frequently found in the stomach more than 24 hours after feeding, which were removed by a stomach pump before recording the tracing.

RESULTS

The normal contractions of the empty stomach were of the type described by Dr. Carlson, the predominating type depending on the length of starvation. As the method used offered no means of differentiating between pyloric and fundic contractions, the results obtained after partial stenosis represent movements of the empty stomach as a whole. Briefly stated they show hypertonicity and hypermotility. The degree of acidity and secretion was not determined in either dog. It might be proper, moreover, to designate here our conception of the use of these terms. By hypertonicity is meant (a) prolonged periods of tetanus beyond the normal—a feature which Carlson and Ginsburg have noted in infants with partial pyloric stenosis and pyloric spasms—and (b) an increase in the strength of the tonic contraction. By hypermotility an increase in the amplitude of the various hunger contractions. Whether marked hypertrophy of the stomach, atony and extreme dilatation would have resulted in these two dogs had the experiments been of longer duration cannot be answered and are points which should be solved.

It is thus clear that partial stenosis of the pylosus induces a hypermotility in the stomach irrespective of the presence of food in the stomach cavity. The hypermotility of the stomach during gastric digestion may be a temporary condition induced by the presence of the food and retardation of its passage through the pylorus, a condition similar to that of the small intestine above a region of obstruction. The fact that the hypermotility is present even in the empty stomach seems to show that the motor changes following mere mechanical obstruction of the pyorus are more fundamental and permanent.

The mechanism of this increased motility can as yet be only conjectured. Bacterial toxins from local foci of infection are excluded in these experiments, as the partial stensosi was produced aseptically in animals with normal stomachs and without subsequent infection. Dr. Carlson suggests nervous reflexes from the pylorus involving the entire stomach. Since the contractions of the empty stomach are primarily automatic, and in no case induced by the stimulation of the nerves in the gastric mucosa, it would seem that if the hypermotility obtained in these experiments is due to any chemical substance arising in the stomach its action on the Auerbach's plexus is probably via the blood.

An experimental animal or a patient, then, retains food longer in

his stomach than normal, if the pyloric sphincter be sufficiently occluded. With this he may suffer from hypermotility, hypertonicity, etc. And since our work shows that these conditions are present even when the stomach is empty, such an individual should, a priori experience more persistent and vigorous hunger pangs, if, as it has been shown, the sensation of hunger runs parallel to the vigor of stomach motility in the normal person at least. As this is apparently not the case, there is evidently a change in the sensory nervous mechanism of the stomach parallel with the motor and secretory changes.

It is interesting to note that tetanic contractions of the empty stomach obtained from dogs with partial pyloric stenosis closely resemble those taken by Luckhardt (18) on dogs during the severe hunger of experimental pancreatic diabetes.

CONCLUSIONS

1. Partial pyloric stenosis in dogs produces hypertonicity, and hypermotility of the empty stomach, even if, of but a few days or weeks duration. These motor phenomena closely simulate those seen in man (filled stomach) with pathological lesions of the pylorus.

2. The same conditions which lead to hypermotility, etc., during digestion, lead at the same time to increased motility of the empty stomach. In other word, partial pyloric stenosis appears to produce a neuro-muscular hyperactivity, independent of the presence of food in the stomach.

My deepest thanks are due Dr. Carlson for his keen interest and timely suggestions during the progress of this work.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXX. The Tonus and Contractions of the Empty Stomach of Infants with Congenital Pyloric Stenosis, Pylorospasm and Chronic Vomiting (Merycism).

A. J. CARLSON and H. GINSBURG

From the Presbyterian Hospital, and the Hull Physiological Laboratory of the University of Chicago

Received for publication November 15, 1915

Pylorus spasm has been ascribed to a great variety of causes, including primary neurosis of the local motor mechanisms. The hyperperistalsis of the filled stomach, usually associated with spasms of the pylorus, may be a temporary condition due to the presence of food in the stomach. On the other hand, if pylorus spasm is simply an expression of primary hypermotility of the entire stomach, this condition of hypertonus and hypermotility should also be in evidence when the stomach is empty. A study of the motor conditions of the empty stomach may thus aid in determining some of the factors involved in hypercontractility of the pyloric sphincter.

In infants "rumination" is probably always secondary to chronic vomiting, which in turn may or may not be associated with pylorus spasm. If the vomiting is due to gastric hypertonicity and hypermotility, these conditions should also be present in the empty stomach, with or without the involvement of the pylorus.

Through the courtesy of Dr. C. G. Grulee we have been able to study the motor conditions of the empty stomach in two infants, one with congenital pyloric stenosis, and one with pylorus spasm, chronic vomiting and rumination. The results on these two cases are reported, not because we feel justified in concluding that the conditions found are typical, but rather with the hope that the work may be extended by others wherever such cases are at hand.

Case 1. Infant 3 months old, chronic vomiting and gradual loss of weight. Congenital pyloric stenosis. Gastro-enterostomy was made. The pylorus was found strongly contracted, and somewhat edematous and anemic. Before the operation record of the tonus and contractions of the empty stomach was made

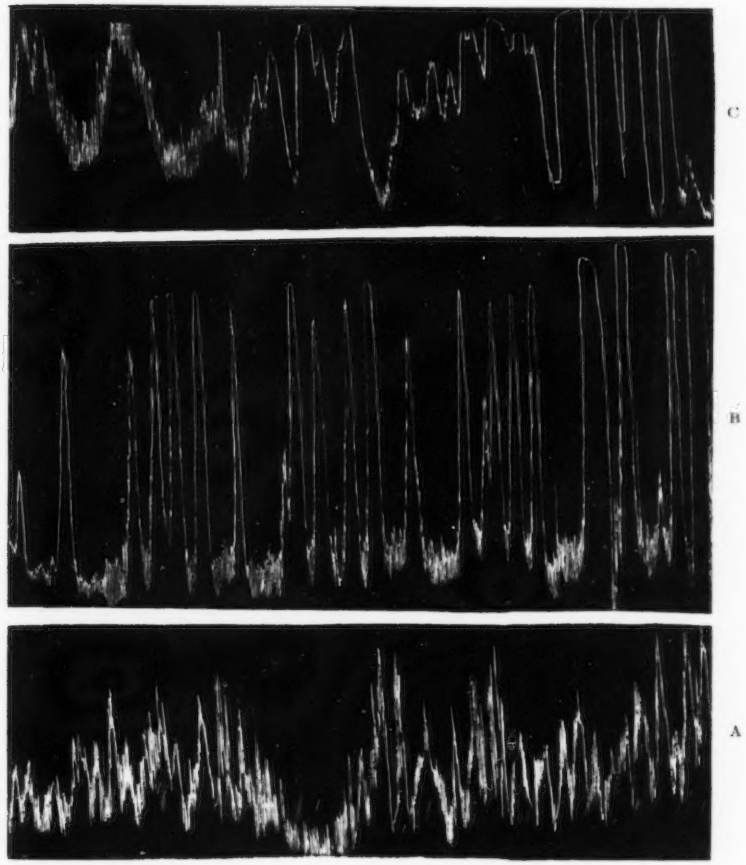

Fig. 1. *A*, Tracing showing a period of vigorous hunger contractions of the empty stomach of a normal infant. *B*, Tracing showing exceptionally intense and practically continuous hunger contractions of a 3-months-old infant with persistent pylorus spasm amounting to almost complete pyloric obstruction accompanied by chronic vomiting and gradual loss of weight. *C*, Tracing showing exceptionally intense hunger contractions and periods of incomplete tetanus of the empty stomach of a five-months-old infant with chronic vomiting ("rumination") and practically stationary body weight. In the right half of the tracing the upward excursion of the manometer had to be checked mechanically to prevent the chloroform from being driven out. Hence the extreme vigor of the gastric contractions are not fully registered. Chloroform manometer. Time, 20 minutes.

by our balloon method as applied to infants. The type of contractions shown by the empty stomach of this infant is shown in figure 1 B. The strength of the contractions was markedly greater than in normal infants. The duration of the periods of contraction was also greater. This indicates a greater than normal gastric tonus. There was no indication of prolonged tetanic contractions.

Case 2[1]. Infant 5 months old, chronic vomiting ("rumination"). Practically stationary body weight. Pylorus spasm. A number of observations were made on this infant. When the child was quiet, so that all nervous inhibitory factors were eliminated the empty stomach usually showed the type of contractions reproduced in figure I C, that is, hypertonus with periods of tetanic contractions lasting several minutes, interspersed with vigorous contractions of normal duration, an unmistakable condition of hypertonicity and hypermotility. If the infant was asleep during the observation period the tetanic contraction of the stomach invariably caused restless facial grimaces or he would wake up and cry. Such vigorous and prolonged period of tetanic contractions have so far never been observed in the empty stomach of normal infants. They have been observed in adult persons and in dogs after prolonged starvation. This type of contractions of the empty stomach may also be seen in dogs with pancreatic diabetes.

The results on these two infants indicate that pylorus spasm and pyloric stenosis involves either primarily or secondarily a condition of hypertonus and hypermotility of the entire stomach. The excessive contraction of the pylorus may be an expression of this general hypermotility. It is known that the tonus and contractions of stomachs in the young mammal are greater than in the adult and in the old. This may be correlated with the greater tendency to hyperactivity of the pylorus in infancy.

[1] Other details of this case reported by Dr. Strauch, Journ. Am. Med. Asso., 1915, lxv, p. 678.

THE RESISTANCE OF FRESH WATER FISH TO CHANGES OF OSMOTIC AND CHEMICAL CONDITIONS

WALTER E. GARREY

From the Physiological Laboratory of Washington University, St. Louis

Received for publication November 17, 1915

The effects of varying the concentration and character of the media in which marine fish live have been the subject of much searching inquiry. Fresh water fish, on the other hand, have been subjected in this connection to only very casual experimentation; P. Bért, (1) for example, investigated the cause of death of fresh water sticklebacks (Gasterosteus leiurus) when put into undiluted sea water. In his experiments some individuals lived only two hours, while others survived a month or more—a variability unexplained by him. R. Florentine (2) could not reconcile his results with those of Bért. Although he worked with the same species, caught in a brook near Nancy, he states that no individual fish lived in undiluted sea water more than six hours. Semper (3) states that

> The common stickleback, Gasterosteus aculeatus, which usually lives in fresh water, lives and thrives perfectly in the Bay of Kiel as well as in the North Sea, and specimens of this fish, caught at Würzburg in the month of May, were even placed at once in sea water without sustaining any injury.

Giard (4) made repeated transfers from fresh to sea water and back, with a daily interval, without perceptibly impairing the vitality of the fish, but he worked with a brackish water stickleback (Gasterosteus trachurus). None of these investigations took into consideration the actual quantitative conditions of osmotic pressure, either of the blood of the fish, or of the solutions used. In view of the contradictions and of the fact that the osmotic relationship between the blood and the natural external medium is, for fresh water fish, the reverse of that existing between the blood of marine teleosts and the sea water, it has seemed advisable to experiment with fresh water fish somewhat more systematically than has previously been done.

In selecting material for this work we have purposely avoided possible confusion with the issues of adaptation and adjustment such as natur-

ally arise when working with fish which live in brackish water, or which migrate between fresh and salt water. We have chosen a small hardy form (Notropis blennius, Girard),[1] breeding and living throughout the year in the Mississippi and Missouri rivers, several hundred miles from salt water.

Preliminary to these studies the osmotic pressure of the blood of a number of species of Mississippi river fish was determined by the freezing point method (5). The results conformed very closely to those obtained for other species by previous observers; all showed the freezing point of blood of these fresh water fish to be very nearly −0.50° C., corresponding to an osmotic pressure of approximately six atmospheres. Since the adult fish selected for experimentation are only four to six centimeters in length it was inexpedient to determine the osmotic pressure of their blood directly. Although microscopic examinations of the corpuscles were made after treatment with solutions of various concentrations, the results were not altogether satisfactory, but they led us to believe that we are correct in the assumption that the blood has the same osmotic pressure as that found for all other fresh water teleosts so far investigated. Our experiments strengthen our belief in the correctness of this assumption.

It was found most advantageous to test the lethal action of the solutions. The longevity was determined by placing only two or three fish in a partially covered glass finger bowl containing 100 cc. of the solution. The solution was changed daily. By this procedure control fish were kept without mortality in tap water, at the laboratory temperature, for more than two months. No food was administered during this period, and no fish were experimented with which had not been in the laboratory aquarium at least two weeks.

In the earlier, winter experiments difficulty was experienced in transferring the animals from the cold tap water running into the aquarium (5°–10°C.): the sudden transfer to a solution or even to tap water at the laboratory temperature (22°–27°C.) resulted in immediate death of many of the fish (cf. Loeb and Wasteneys) (6). This element of uncertainty was eliminated by allowing the temperature to rise gradually during several hours. The fish were then kept in tap water at the higher temperature for two days before use. Before subjecting them to solutions, they were washed by transferring to distilled water for one-half hour.

[1] I am indebted to Prof. J. F. Abbott for the identification of this fish.

LONGEVITY IN DISTILLED WATER.

Tap water had a distinct saline content; that of St. Louis at the time of these experiments gave a distinct cloudiness due to calcium salts when tested with soluble oxalates, and was strongly alkaline to phenolphthalein. In view of the neutral character of distilled water and in the light of the observations of Ringer (7), (8) concerning the conserving effects, even of small amounts of salts, especially calcium salts, and of alkalis (bicarbonates) it was desirable for purposes of control to determine the longevity in distilled water. In all 31 animals were placed at different times and under variable conditions of temperature, in water redistilled from glass, this distilled water being changed at least daily. Of these, 21 lived, without food, until the experiments were discontinued at which time one fish had lived eight weeks, 8 had survived six weeks, 6 had survived four weeks and 6 had survived three weeks. Of the 10 which did not survive, 4 died in less than twelve hours, death probably being due to lesions which existed prior to the experiment, for they showed distinct abrasions on the body surface especially an inflammatory condition of the tail fin and posterior end of the body.[2] The other 6 fish died within a week, 2 of these being afflicted with the "gas-bubble disease."

Since 68 per cent of our fish survived the action of redistilled water for weeks and since among the 32 per cent which died several individuals were injured or unhealthy, we can hardly look upon this medium as a very toxic one for this species. Loeb has also shown that among marine fish, the adults as well as the embryos of Fundulus heteroclitus live in distilled water. Not even the injured fish of our series were killed with the rapidity reported by Ringer for "minnows, eels, sticklebacks and gold fish," viz., four and one-half to fourteen hours, or by Wells (18) for the white crappie (Pomoxis annularis). None the less, within the time limits of our distilled water experiments, a mortality of 32 per cent was never observed among our stock animals which included injured as well as normal individuals; and this is true whether the fish were kept in the tap water aquarium or in finger bowls. These experiments indicate, as did Ringer's, that tap water contains some

[2] The prompt death of these injured fish is a constant result even in tap water, or in any hypo- or hyper-tonic solution. The fish, however survive much longer in balanced saline solutions isosmotic with the blood and in this respect behave as Fundulus has previously been shown to behave, after scraping the body surface; cf. Garrey, (9). Bért (10) had previously noted that eels with an injured surface died when transferred from fresh water to sea water.

substances (salts) which serve to protect the body surfaces (gills) of the fish. They also add to the evidence that there are very marked differences in the vital resistance offered by different species of fresh water fish to distilled water. A further discussion of the effects of distilled water will be found in the section dealing with alkalinity.

THE INJURIOUS EFFECTS OF CANE SUGAR

The fact that a large proportion of the fish lived well in distilled water to which no salts have been added induced us to try out the effects of cane sugar solutions in which it was conceivable that the effects would be purely osmotic, although this conception proved to be erroneous. The sugar was twice recrystallized in two experiments; in eight others, "highest purity" (Merck) sugar was used and in two series of experiments commercial "Domino" sugar was used. Little difference could be noted in longevity in these different solutions although on the whole the fish lived a negligibly longer time (3–12 hours) in the commercial product. The results of the experiments are summarized in Table I.

TABLE I

MOLAR CONCENTRATION	Δ = C.°	PER CENT DEAD AT END OF			
		12 hrs.	24 hrs.	2 days	3 days
0.1	−0.198	0	33	100	
0.2	−0.397	0	0	33	100
0.25	−0.49	0	33	66	100
0.3	−0.59	33	100		
0.4	−0.77	100			

These experiments demonstrate clearly that the fish die within a few hours (12) in a 0.4 molecular cane sugar solution which has an osmotic pressure about 50 per cent above that of their blood, but live much longer (3 days) in a solution of approximately the osmotic pressure of the blood, e.g., between 0.2 and 0.25 mol.

While the influence of osmotic pressure is clearly demonstrated in the increased mortality of the fish in solutions with concentrations above that of their blood, other factors were clearly involved, for the fish died sooner in $\frac{M}{10}$ cane sugar solutions than in either distilled water or 0.2 mol. solutions of cane sugar.

The results tabulated above clearly show that cane sugar solutions are, either directly or indirectly, toxic. This toxicity can be decreased

by increasing the osmotic pressure of any hypo-osmotic solution of this sugar until it is equal to that of the blood, but above this concentration, which seems to be optimum, a further increase in the osmotic pressure increases, progressively, the toxicity of the solution.

Bért ascribed the effects of changing the concentration of media to which fish were subjected, to a primary injury to the gills, as he stated it, "a physico-chemical alteration in the properties of the (gill) membranes." He also described a "very congested" condition of the gills due to the action of sea water. Ringer, Loeb, and also Herbst (11) point out the probability of a change in permeability of the gills as the event of primary importance. J. Loeb (12) has more recently attributed the increased permeability of the gills of Fundulus in sugar solutions, in part at least, to fermentative acid formation. That acid is a factor he demonstrated conclusively, but he also observed a direct toxic action of the sugar solutions. His experiments demonstrated a greater death rate in cane sugar than in isotonic dextrose solutions, which we believe would be difficult to understand if the only factor at work was that of acidity. The direct toxic action of sugar in experiments with Fundulus was noted by Loeb in solutions more concentrated than $\frac{M}{4}$. This concentration is hypertonic for fresh water fish. As a result, possibly of the membrane changes due to the action of hypertonic sugar solutions upon the gills, the osmotic extraction of water doubtless becomes a factor in increasing the toxic effects (even for Fundulus). That the mortality in solutions of cane sugar is, however, not primarily an osmotic effect, but must be referred to some other mechanism is demonstrated in our experiments by the fact that the fish live much longer in certain saline mixtures of equivalent or even greater osmotic pressures. Similarly, taking advantage of the fact which Loeb found, in this connection, viz., a distinct antagonism of mixtures of salts for the toxic effects of cane sugar solutions, we have found that in a mixture of equal parts of $\frac{M}{4}$ cane sugar and a weak Ringer's solution ($\Delta = 0.46°C.$) the fish lived more than two weeks, while in $\frac{M}{8}$ cane sugar solution which therefore had the same sugar content, but only half the osmotic pressure of the mixture, the fish did not survive the third day. All solutions were freshly made and changed daily.

In the action of cane sugar upon these fresh water fish, our results deviate in only one particular from those obtained by Loeb for the marine form, Fundulus, viz., the greater toxicity of weaker than of the more concentrated sugar solutions when both are hypotonic. It seems hardly plausible to attribute this result to a greater acidity of

the weaker solutions or to a lower toxicity of the more concentrated solutions. Leo Loeb (22) has described marked changes in the surface and cytoplasm of blood cells of Limulus due to sugars. In the case of fish the toxic factors would seem to be such as to increase the permeability of the surface or gill membranes and thus to introduce the play of osmotic forces which previously had been ineffective. In consequence the tissues and body fluids of the fish take up water from hypotonic solutions, the more dilute the more rapidly, but are spared the rapid dilution (and possibly the loss of salts) by increasing the concentration of the external medium. It should be added that the experimental results exclude hyperglycemia (saccharosemia) as the cause of the toxic action of cane sugar.

THE TOXICITY OF DILUTED SEA WATER

The experiments of Bért indicated clearly that, had it been used in proper concentration, sea water would not have been very toxic for fresh water fish, and it has since been amply demonstrated that for tissues of a great variety of forms diluted sea water must be looked upon as a very well "balanced solution" (13). It was therefore deemed probable that it could be used as a control with which to check up the osmotic action of other solutions. The effects of various dilutions of sea water upon the longevity of our fresh water fish are shown in Table II.

TABLE II

	CONCENTRATION	Δ=C°	LONGEVITY
1	Undiluted sea-water (Woods Hole)	−1.81	20 minutes or less
2	75 cc. sea-water + 25 H_2O (dist.)	−1.35	20 minutes
3	60 cc. sea-water + 40 H_2O (dist.)	−1.09	25 minutes to 40 minutes
4	50 cc. sea-water + 50 H_2O (dist.)	−0.915	1 hr. 5 min. to 1 hr. 45 min. (average 1 hr. 15 min.)
5	45 cc. sea-water + 55 H_2O (dist.)	−0.82	2 hrs. to 6 hrs. (average 4 hrs.)
6	40 cc. sea-water + 60 H_2O (dist.)	−0.73	Min. 4 hrs., max. 20 hrs. (average 12 hrs.)
7	35 cc. sea-water + 65 H_2O (dist.)	−0.64	Average 2 weeks, max. 5 weeks
8	30 cc. sea-water + 70 H_2O (dist.)	−0.547	6 weeks—experiments discontinued.
9	In all more dilute sea-water the fish lived as well as in tap water.		

The solution containing 30 cc. sea water diluted with 70 cc. of distilled water has a freezing point of 0.547°C. and is only very slightly hypertonic for the blood. At this concentration the curve of longevity takes a sharp turn. The isotonic sea water was not more detrimental than the more dilute sea water or than tap water, and less harmful than distilled water under the best of laboratory conditions. An

isotonic solution may thus be regarded as having the "critical" concentration, since more concentrated solutions were rapidly and progressively more detrimental as the concentration increased. Bért went so far as to state that the sole cause of death of *frogs* in sea water is attributable to the desiccation of blood and tissues. He noted a loss of weight by fish as well as by frogs which amounted to $\frac{1}{5}$ or even $\frac{1}{3}$ of the body weight; Loeb and Wasteneys (14) have recently added some interesting data on the osmotic changes of Fundulus in various solutions.

We have demonstrated that in the case of hypotonic solutions the mixture of salts in the proportion in which they are found in sea water is non-toxic for the fresh water fish with which we have experimented, and that the deleterious effects of the hypertonic solutions are mainly osmotic and proportional to their water extracting proclivities.

The lack of resistance of our fresh water fish, when placed in sea water which is hypertonic for their blood, is in interesting contrast to the resistance which marine teleosts exhibit, for they normally are not affected by the hyperosmotic sea water in which they live.

THE TOXICITY OF SODIUM CHLORIDE

In striking contrast to the longevity of the fish in diluted sea water is their mortality in weak solutions of sodium chloride as shown in Table III.

TABLE III

PER CENT NaCl	Δ = °C.	LONGEVITY			COMPARATIVE LONGEVITY IN SEA WATER OF SAME OSMOTIC PRESSURE
		Maximum	Minimum	Average	
1.35	−0.8	1 hr. 46 min.	50 min.	1 hr. 15	2 hrs.
0.9	−0.54	3 days	30 hrs.	2 days	14 days
0.76	−0.46	15 days	3 days	4 days	6 weeks+
0.6	−0.37	15 days	4 days	12 days	6 weeks+
0.45	−0.275	23 days		15 days	6 weeks+
0.25	−0.105	24 days	9 days	18 days	6 weeks+

It was noted above (Table II) that in sea water iso-osmotic with the blood of these fish, and in all more dilute sea water, the experimental animals survived the duration of the experiment (6 weeks). Table III shows that in sodium chloride of this concentration (0.76 per cent) they did not survive a week. Although little difference is to be noted

between the toxicity of 0.45 per cent and 0.25 per cent solutions of sodium chloride, there is a progressive increase in the toxicity with increasing concentration, until, at that of the blood, i.e., at the "critical concentration," the toxicity curve takes a sudden turn and the mortality of the animals increases at a very rapid rate. The slow increase in mortality with the increase in the concentration of the hypotonic solutions is very nearly proportionate to the mass action of the sodium chloride, but the change in the mortality curve shows that a new lethal factor has been introduced at the "critical concentration." This factor is superimposed upon the toxic action of the salt (NaCl), and is, we believe, osmotic in character. This view is strengthened by the fact that with the stronger (hypertonic) solutions the differences between the toxicity of NaCl and of sea water are not striking; although sodium chloride was on the average the more toxic, the variability in the longevity of individual animals was such as to make any quantitative statement of this conclusion unsafe.

The non-toxic character of hypotonic sea water—a "balanced solution"—as contrasted with the striking toxicity of equivalent concentrations of sodium chloride, pointed to these fish as especially favorable material for the study of the antagonistic action of salts, and work was extended along these lines.

CALCIUM CHLORIDE

We will record first a summary of the results obtained with pure solutions of calcium chloride.

In $CaCl_2$ ($\frac{M}{20}$) the fish lived only 3.5–6 hours.
In 50 cc. $CaCl_2$ ($\frac{M}{20}$) + 50 cc. H_2O (dist.) the fish lived 2–4 days.
In 25 cc. $CaCl_2$ ($\frac{M}{20}$) + 75 cc. H_2O (dist.) the fish lived 4–20 days.
In 15 cc. $CaCl_2$ ($\frac{M}{20}$) + 85 cc. H_2O (dist.) the fish lived 14–21 days.
In 5 cc. $CaCl_2$ ($\frac{M}{20}$) + 95 cc. H_2O (dist.) the fish lived 5–7 weeks (exp. discontinued).

In this series of experiments the calcium chloride was found to be much more toxic than sodium chloride of the same concentration; for example, the longevity in $\frac{M}{40}$ $CaCl_2$ (2–4 days) is not greater than in $\frac{M}{8}$ NaCl in spite of the higher osmotic pressure of the latter solution.

MIXTURES OF SODIUM AND CALCIUM CHLORIDE

The well-known antagonism of calcium salts for the toxic effects of sodium chloride was forcibly illustrated in experiments of which the following series is typical.

67 cc. NaCl (1.35 per cent) + distilled water to 100 cc. (=0.9 per cent NaCl) fish lived 2 days.

67 cc. NaCl (1.35 per cent) + 0.5 cc. $CaCl_2$ ($\frac{M}{10}$) + dist. H_2O to 100 cc. fish lived 4–8 days.

67 cc. NaCl (1.35 per cent) + 1.0 cc. $CaCl_2$ ($\frac{M}{10}$) + dist. H_2O to 100 cc. fish lived 8–10 days.

67 cc. NaCl (1.35 per cent) + 5.0 cc. $CaCl_2$ ($\frac{M}{10}$) + dist. H_2O to 100 cc. fish lived 8–10 days.

Adding 10, 15, 20 and 25 cc. of $CaCl_2$ ($\frac{M}{10}$) to the 0.9 per cent sodium chloride did not produce any more favorable results, nor did the presence of these larger amounts of calcium perceptibly increase the toxicity. The experiment illustrates how little calcium is necessary to produce a marked decrease in the toxicity of sodium chloride. The reciprocal antagonism of the salts of sodium for those of calcium is also revealed, for experiment showed that the fish live 8 or 10 days in a mixture of 75 cc. NaCl (0.9 per cent) + 25 cc. $CaCl_2$ ($\frac{M}{10}$). This solution contains $\frac{M}{40}$ $CaCl_2$, in pure solutions of which the fish lived only 2–4 days. Sodium chloride, thus, decreased the toxicity of the $CaCl_2$. The mixture of these two salts is not, however, as favorable a medium for these fish as is sea water of the same concentration. In the hope of determining the rôle of the other salts in producing the physiological balance of sea water, the experiments were continued and the toxicity of the salts and their mixtures in various proportions was tested.

POTASSIUM CHLORIDE

Solutions of pure potassium chloride proved more toxic than similar solutions of the equi-osmotic solutions of the chlorides of either sodium or calcium, as is seen in the following experiment.

KCl ($\frac{M}{10}$)	the fish died in 3- 5 hrs.
KCl ($\frac{M}{10}$) 50 cc. + H_2O distilled, 50 cc.	the fish died in 3- 8 hrs.
KCl ($\frac{M}{10}$) 25 cc. + H_2O distilled, 75 cc.	the fish died in 12-18 hrs.
KCl ($\frac{M}{10}$) 15 cc. + H_2O distilled, 85 cc.	the fish died in 20 hrs.
KCl ($\frac{M}{10}$) 5 cc. + H_2O distilled, 95 cc.	the fish died in 12-29 hrs.

MIXTURE OF NaCl + KCl

In a mixture of 95 cc. $\frac{M}{9}$ NaCl + 5 cc. $\frac{M}{10}$ KCl the fish lived two or three days. Here again, we find that sodium chloride has in some measure served to protect the animals from the toxic action of KCl, just as it did from $CaCl_2$, a result which is evident in spite of the fact

that the osmotic pressure was practically at the limit of the animals' tolerance.

The reciprocal action by which the toxicity of sodium chloride would be decreased by potassium chloride was not revealed in our experiments as it was in those of Loeb and Wasteneys (15) with Fundulus. Our animals did not live longer in any mixture of sodium and potassium chloride than in pure sodium chloride of the same osmotic pressure. The addition of progressively increasing amounts of potassium chloride from 1 cc. to 25 cc. per 100 cc. of the mixture caused a progressive shortening of the period of survival of the fish. It is possible that we were working with concentrations of NaCl which were too great and that success in this direction may attend a different mode of attack.

MIXTURES OF POTASSIUM CHLORIDE AND CALCIUM CHLORIDE

Calcium chloride reduces markedly the toxicity of potassium chloride for these fish, as is illustrated in the following series of experiments.

5 cc. KCl ($\frac{M}{10}$) + 95 cc. H_2O distilled — Longevity 24 hrs. (average).
5 cc. KCl ($\frac{M}{10}$) + 1 cc. $CaCl_2$ ($\frac{M}{20}$) + H_2O distilled to 100 cc. Longevity 6 days 1 lived 13 days).
5 cc. KCl ($\frac{M}{10}$) + 15 cc. $CaCl_2$ ($\frac{M}{20}$) + H_2O distilled to 100 cc. Longevity 9 days.
15 cc. KCl ($\frac{M}{10}$) + 15 cc. $CaCl_2$ ($\frac{M}{20}$) + H_2O distilled to 100 cc. Longevity 13 days
15 cc. KCl ($\frac{M}{10}$) + 0 + H_2O distilled to 100 cc. Longevity 20 hrs.
25 cc. KCl ($\frac{M}{10}$) + 0 + H_2O distilled to 100 cc. Longevity 12-18 hrs.
25 cc. KCl ($\frac{M}{10}$) + 5 cc. $CaCl_2$ ($\frac{M}{20}$) + H_2O distilled to 100 cc. Longevity 36 hours.
25 cc. KCl ($\frac{M}{10}$) + 15 cc. $CaCl_2$ ($\frac{M}{20}$) + H_2O distilled to 100 cc. Longevity 36 hours.
25 cc. KCl ($\frac{M}{10}$) + 25 cc. $CaCl_2$ ($\frac{M}{20}$) + distilled to 100 cc. Longevity aver. 36 hours (one animal lived 7 days).

In these experiments, as in those with sodium chloride, the decrease in toxicity of KCl is demonstrable even with very small amounts of calcium chloride but the criterion, longevity, does not warrant any interpretation which would fix an optimal ratio for these mixtures. On the other hand, we were not able to affirm from our experiments that there was a decrease in the toxicity of solutions of calcium chloride by the addition of KCl. In all of a large number of experiments to test this point, experiments with a comprehensive range of concentration of both calcium and potassium solutions, the presence of potassium chloride invariably caused death of the fresh water fish sooner than the control solutions containing calcium chloride alone.

Summarizing the results of our experiments with KCl, we may say that for these fresh water fish this salt is more toxic, molecule for

molecule, than either NaCl or $CaCl_2$. Both Na and Ca materially decreased the toxicity of potassium chloride. The reciprocal detoxication by this salt of either Na or Ca was not sufficiently striking, if present, to admit of demonstration by these experiments.

No fact has been more firmly established by experiment than that for a large variety of tissues and organisms, the addition of potassium chloride to mixtures of Na and Ca is favorable to life and function. Further experimentation showed that this can also be demonstrated for Notropis blennius in spite of the contradictory character of the results obtained in the experiments with sodium chloride or calcium chloride alone when KCl was added. It has already been noted that in pure 0.9 per cent NaCl the fish lived only two days at the most. The addition of $CaCl_2$ to the sodium chloride prolonged the life to a maximum of ten days. Fish were at the same time placed in a parallel series containing sodium chloride (0.9 per cent), but to the solution was added $CaCl_2$ (ad .024 per cent) and KCl (ad .042 per cent). This is the proportion which is frequently used in making "Ringer's solution" for experiments with mammalian tissues. The solution had a $\Delta = -0.55°C$. In this solution the minimal duration of life of our fresh water fish was equal to the maximum in sodium chloride solutions with the optimal amount of calcium chloride, while the longest duration of life in this solution was double that in the mixture which contained the other two salts but did not contain the potassium chloride. In passing it may be noted that the amount of potassium chloride in this solution if present in distilled water alone, causes the death of these fish in 24 hours, and that we have here an excellent example of the neutralization of the toxicity of potassium by a mixture of Na and Ca as well as of the fact that the Na-Ca complex is decreased in toxicity by a substance in itself more toxic than the complex, and further that the effect upon the Na-Ca complex is demonstrable even though we could not demonstrate an antagonistic effect upon the individual chemicals of the mixture.

Ringer's solution, slightly alkaline, gives a good example of what is meant by physiological "balance," i.e., *mutual* decrease of toxicity. We know of no tissue for which Ringer's solution is not less toxic than pure sodium chloride solution; it is not, however, the *best* saline solution for the fresh water fish with which we experimented—it is not perfectly balanced. The earlier experiments of our series showed that healthy individuals lived in sea water of the same osmotic pressure as the Ringer's solution (30 cc. of Woods Hole sea water + 70 cc. distilled

water) for six or seven weeks, at the expiration of which time the experiments were discontinued. Out of many individuals tested not one lived more than four weeks in the Ringer's solution and few survived more than two weeks.

THE EFFECTS OF MAGNESIUM CHLORIDE

In attempting to determine why sea water was less toxic than Ringer's fluid, our attention is naturally directed to the large content of magnesium salts which are not present in Ringer's fluid. Almost exactly one-tenth of the molecules of sea water are of magnesium salts (van't Hoff) their concentration is therefore equal to $\frac{M}{20}$ $MgCl_2$. Bért and DeVarigny have both contended that magnesium salts in the concentration in which they are present in sea water are not toxic for fresh water sticklebacks. This is surprising in the face of such results as the following which we obtained with Notropis blennius.[3]

(*a*) $MgCl_2$ ($\frac{M}{10}$) Average longevity 1 hour 10 minutes.
(*b*) $MgCl_2$ ($\frac{M}{10}$) 50 cc. + distilled H_2O 50 cc. Average longevity 20 hours–24 hours.
(*c*) $MgCl_2$ ($\frac{M}{10}$) 25 cc. + distilled H_2O 75 cc. Average longevity 48 hours.
(*d*) $MgCl_2$ ($\frac{M}{10}$) 15 cc. + distilled 85 cc. Average longevity 3–5 days.
(*e*) $MgCl_2$ ($\frac{M}{10}$) 5 cc. + distilled H_2O 95 cc. Average longevity 4–6 days.

Solution (*a*) has approximately the osmotic pressure of the blood of the fish; solution (*b*) has about the concentration of this salt in sea water ($\frac{M}{20}$); solution (*d*) in which the fish live five days at most contains the same content of magnesium chloride ($\frac{M}{60}$) as 30 cc. of sea water diluted with 70 cc. of distilled water in which the fish lived six weeks or more. Obviously magnesium chloride is very toxic, but its toxicity is reduced to a minimum by the presence of the other salts of diluted sea water; conversely we may infer that magnesium decreases the toxicity even of so well balanced a mixture of Na, Ca and K chloride as Ringer's solution.

An objection may be raised to these conclusions, viz., that the presence, in sea water, of anions not present in Ringer's solution, might account for the lower toxicity of the sea water and that the effect

[3] It is worthy of note that the fish in solutions of magnesium chloride show distinct anaesthetic effects for some time before death. They are unable to maintain their equilibrium, lie upon the side or with ventral surface up when at rest, and roll about the long axis when swimming. Respiratory movements of the gill opercula are of the Cheyne-Stokes type.

may not be due to magnesium. To obviate this criticism mixtures of chlorides alone were compared, all in $\frac{M}{10}$ concentration. (*a*) Ringer's fluid: NaCl, 100 cc. + KCl, 2.2 cc. + $CaCl_2$, 2.0 cc. In this solution the fish lived about two weeks. (*b*) Ringer's fluid as in solution (*a*) + 12 cc. $MgCl_2$. In this solution the fish were still alive at the end of five weeks whereupon the experiment was discontinued. The magnesium chloride then antagonized the toxicity of the neutral Ringer's fluid to a marked degree although the concentration of magnesium was such that in the absence of the other salts, it would have killed the fish in four or five days. The above criticism then is not valid, and the antagonism noted with sea water was due to the presence of magnesium salts.

Loeb has demonstrated, unequivocally, the antagonistic action of magnesium salts for the toxic action of sodium salts. We were astonished to find that no such antagonism was demonstrable in our series of experiments directed to this end. In fact the addition of $MgCl_2$ to NaCl always increased the toxicity of the solution, acting in this respect like KCl. The contrast between this failure and the marked conserving action exerted by magnesium salts upon the mixtures of Na + K + Ca chloride would seem to point to an antagonism which must be referred to an effect exerted in the main to decrease the toxicity either of calcium or of potassium, or of their combined effect—which may be different from that of either salt singly.

MIXTURES OF MAGNESIUM CHLORIDE WITH POTASSIUM CHLORIDE

A very distinct antagonism of $MgCl_2$ for $\frac{M}{10}$ KCl was observed as is illustrated in the following series. (*a*) In 15 cc. KCl ($\frac{M}{10}$) + 85 cc. distilled water the average longevity was 20 hours and the maximal 48 hours (one fish only). (*b*) In 15 cc. KCl $\frac{M}{10}$ + 80 cc. H_2O + 5 cc. $MgCl_2$ ($\frac{M}{10}$) the minimal duration of life was 70 hours and the maximal between 5 and 6 days, showing the conserving action of the magnesium salt. (*c*) In the same concentration of potassium chloride but with 15 cc. of $MgCl_2$ ($\frac{M}{10}$) the first death occurred in 28 hours, the average longevity was 72 hours and the maximal just under four days. The fish still lived longer than in the pure solution of KCl, but not so long as with the smaller amount of magnesium chloride. Increasing the content of the magnesium salt above that in solution *c*) caused a progressive increase in toxicity. (*d*) When the solution contained 25 cc. of $MgCl_2$ ($\frac{M}{10}$) the fish lived only two or three days, but the solution was

less toxic than the pure KCl solution without Mg. The solution was more toxic than one containing magnesium chloride alone, showing that the toxicity of the magnesium salt had not been decreased by the presence of KCl in any of the concentrations used.

MAGNESIUM VS. CALCIUM

Loeb (16) demonstrated that the continued contraction of the muscles of Polyorchis, which appears in solutions containing calcium, is antagonised by magnesium with resultant relaxation; and Meltzer and Auer (17) showed that the anaesthetic action of magnesium sulphate was neutralized by calcium chloride. Concerning these reciprocal antagonisms, the following data were obtained with mixtures of $\frac{M}{10}$ solutions of the chlorides of magnesium and calcium.

15 cc. $MgCl_2$		+85 cc. H_2O	Lived 4 days.
15 cc. $MgCl_2$ +	5 cc. $CaCl_2$ +	80 cc. H_2O	Lived 5–7 days
15 cc. $MgCl_2$ +	15 cc. $CaCl_2$ +	70 cc. H_2O	Lived 6–7 days.
25 cc. $MgCl_2$ +	0 cc. $CaCl_2$ +	75 cc. H_2O	Lived 2 days.
25 cc. $MgCl_2$ +	10 cc. $CaCl_2$ +	65 cc. H_2O	Lived 6 days.
25 cc. $MgCl_2$ +	25 cc. $CaCl_2$ +	50 cc. H_2O	Lived 6 days.
	25 cc. $CaCl_2$ +	75 cc. H_2O	Lived 4–6 days.

Calcium chloride unquestionably decreased the toxicity of magnesium chloride. Concerning the reciprocal relation of Mg to Ca: the presence of magnesium at least did not increase the toxicity of the calcium chloride although the osmotic pressure was raised by its addition, and in the last figures given there is an indication of some decrease in the toxicity of $CaCl_2$ due to the addition of $MgCl_2$.

ALKALINITY

Some of the facts which have developed in the course of this investigation are not without significance in view of the recent generalization of Wells (18) that "fresh water fish cannot live normally in water that is alkaline but require a certain degree of acidity to carry out their normal activities." The fish with which we have worked have been kept in our laboratory for months in the running tap water; they and many other species are similarly kept in the aquaria of the dealers in fish bait, and in other aquaria about the city of St. Louis. The city water of St. Louis is quite strongly aklaline owing to the method of purification of the Mississippi river water by precipitation with lime and ferrous sulphate. It gives an alkaline reaction to phenolphthalein.

rosolic acid, neutral red, congo red, methyl violet and litmus. It shows a distinct titratable alkalinity as shown by the reports of the chemist. Sörensen's colorimetric methods in our hands showed the concentration of $\overline{OH}$ to be somewhat more than $N \times 10^{-5}$.

In aquaria plentifully supplied with this running water the alkalinity was not perceptibly altered by the presence of the fish; neither the body excretions nor CO_2 developed by the fish had any effect in decreasing the alkalinity of the aquarium water.

The fish lived for weeks in all dilutions of sea water less concentrated than their blood. Woods Hole sea water was used and this has been found by Loeb (19) to have an alkalinity somewhat greater than $\overline{OH} \times 10^{-5}N$. The stock Ringer's solution of our laboratory contains 0.03 per cent sodium bicarbonate and we have noted a minimum longevity of two weeks in this solution. This solution was rendered much less toxic for our fish by the addition of magnesium chloride which, in this saline mixture, did not alter the hydroxyl ion content.

The presence of neutral salts (properly balanced) in the above solutions may have decreased the toxicity of the hydroxyl ions; while this possibility should be further investigated, Ringer's (7) experiments, which we have confirmed, showed that bicarbonate of sodium (0.03 per cent) alone decreased the toxicity of distilled water for fresh water fish.

As to the necessity of acidity, the above experiments speak conclusively against its requirement by the species of fish with which we worked. Of further evidence in this direction it should be noted that the animals could be kept for considerable periods in distilled water and much longer in balanced mixtures of the neutral chlorides. The only possibility of acid development in these solutions lies in the accumulation of CO_2 excreted by the animals themselves. This accumulation of course, cannot be entirely eliminated by the finger bowl method; it was reduced to a minimum, however, by frequent changes of water and by the free access to the air and agitation which was obtained in all experiments by the movements of the fish, and in some by bubbling alkali-washed air through the solutions. In none of the experiments was the sea water or alkaline Ringer's fluid rendered acid by any such accumulation of CO_2. Finally experiments were made in which distilled water and neutral salt solutions were changed by a continuous stream and constant level device so that double the volume of the fluid was replaced each hour. The animals were alive at the end of three weeks when the experiments were discontinued.

Alkali in the form of sodium bicarbonate was found by Wolfgang Ostwald (20) to prolong the life of fresh water Gammarus when placed in saline solutions and our experience indicates that this is true also for the fresh water fish (Notropis blennius). This result was to be expected for Gaule (21) in 1878 showed that alkali was necessary to prevent the development of acidity by the beating heart. Since that time the presence of a certain excess of free $\overline{OH}$ ions along with available alkalinity has been found to be advantageous to the functional activity of tissues by all physiologists who have worked with conservative fluids (Ringer, Langendorf, Locke, Tyrode, Clark).

That differences in resistance of various species of fresh water fish to alkalis should be found is not surprising, similar differences have been noted by J. Loeb (19) between such closely related marine forms as Strongylocentrotus purpuratus and Arbacia punctulata. The eggs of the former begin development only in the presence of alkali, while those of the latter will begin but not continue development in neutral or even faintly acid solutions; even for the latter the optimal fluid contains free OH ions in the concentration in which they are present in Woods Hole sea water ($C_{OH} = > N \times 10^{-5}$.)

On the whole it may be said that, except for osmotic and concentrational differences, the resistance of fresh water fish to salts and their mixtures is quite like that of marine fish. It therefore seems futile to attribute the experimental results with either form to special adaptation or adjustment.

SUMMARY

1. The fresh water fish, Notropis blennius, will live months in the tap water of St. Louis, which is distinctly alkaline. These animals also live in redistilled water for weeks.

2. For these fish cane sugar solutions are directly toxic, least so if the osmotic pressure is equal to that of the blood. This toxicity is reduced by salts and alkalis.

3. Individually the solutions of chlorides of potassium, magnesium calcium and sodium are toxic. In equivalent concentrations the relative toxicity is K>Mg>Ca>Na.

4. Sodium chloride decreased the toxicity of chlorides of Ca, Mg and K. Calcium chloride decreased the toxicity of chlorides of Na, K and Mg. Magnesium chloride decreased the toxicity of chlorides of Ca and K but not that of sodium. Potassium chloride did not decrease the toxicity of any single salt (Ca, Na or Mg).

5. In combinations of two or more of the other salts the antagonistic decrease of toxicity by K and by Mg was apparent. Thus mixtures of the salts demonstrated their mutual antagonism and became progressively less toxic when to sodium chloride was added calcium chloride then potassium chloride and finally magnesium chloride.

6. Sea water diluted to the concentration of the blood (or less) was a perfectly balanced solution for the fresh water fish.

7. When the salts are properly balanced the fresh water fish tolerated an osmotic pressure of the external medium equal to their own blood. Above this concentration, which is "critical," death is prompt.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE EMPTY STOMACH

XXXII. The Effect of Dreaming on the Gastric Hunger Contractions

ARNO B. LUCKHARDT

From the Physiological Laboratory of the University of Chicago

Received for publication, November 29, 1915

During the course of experiments on the movements of the empty stomach of dogs I have noted on several occasions an inhibition of the gastric hunger contractions brought about probably by cerebral influences in the dreaming state during deep sleep. These observations I wish to record in a brief note chiefly because they show that even during sleep emotional states (whether pleasant or unpleasant) may influence bodily states.

The author does not assume that such or similar observations have not been made before. Most of us have experienced so-called night mares with the result that we have awakened ourselves from sleep by our own cry of despair or fright. Other dreams lead to the speaking of a few coherent or incoherent sentences. Or, as the result of emotional stress brought on by pleasant or unpleasant dreams, persons break out into a fit of laughter or wake up weeping. It would seem that the nature of the emotion effects the same bodily change or leads to the same bodily activity irrespective of whether the body is in a waking or sleeping state.

Every form of afferent stimulus in the dog whether agreeable (sight of food, sight of a favorite attendant) or disagreeable (pain, sight of a natural enemy as the cat) leads promptly in the waking state to a diminution in tone of the empty stomach or temporary cessation of the hunger contractions.[1] Pleasure, pain, or fear leading to diminished activity of the empty stomach in the waking state, the same emotional state occurring during sleep might lead to the same inhibition.

It is a simple matter to determine whether or not a dog is asleep. It is more difficult to be certain that dogs dream in the sleeping state.

[1] Carlson: This Journal, 1913, xxxii, 369.

If the dogs, after lying in my lap for some time at ease and amid perfectly quiet surroundings with closed eyes and a regular and slow or slow and stertorous respiration (snoring) would suddenly begin to wag tail, breath more deeply and irregularly, move more or less coördinately fore and hind limbs, show teeth, snarl, or emit abortive yelps, I assumed that the dog was experiencing during sleep a form of cerebral excitation akin to or identical with the dreaming state in man.

METHODS

The method used to obtain contractions of the empty stomach was the same as used in all previous experiments recorded in this series of papers.[2]

RESULTS

Three figures will suffice to show graphically the effect of cerebral excitement during the sleeping state on the contractions of the empty stomach in dogs.

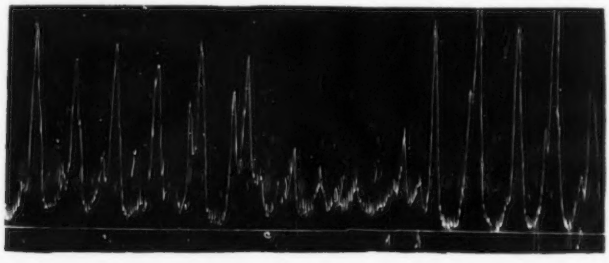

Fig. 1.

Figure 1. During the entire experiment the dog lay in my lap asleep. For $\frac{3}{4}$ of an hour dog showed Type II contractions as shown prior to "*c*" on the tracing. At "*c*" dog's hind legs twitched and jerked about incoördinately. This continued for some time. As can be seen from the tracing there is almost a complete cessation of the stomach contractions which persists for some time before the regular Type II rhythm reappears.

Figure 2. Prior to "*a*" the dog was in my lap sleeping peacefully. As can be seen from the tracing the contractions of the empty stomach

[2] Carlson: This Journal, 1913, xxxii, 369.

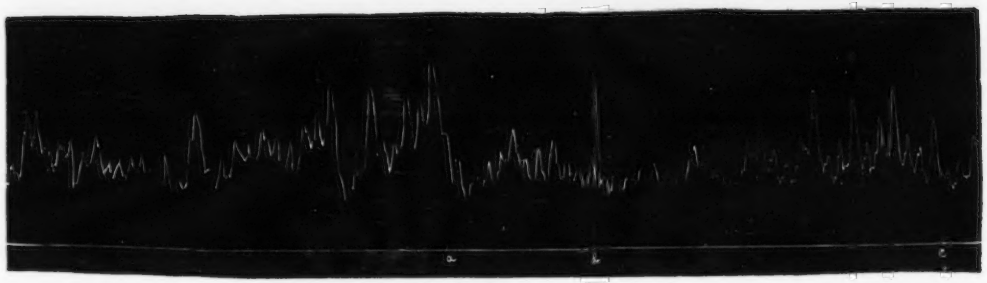

Fig. 2.

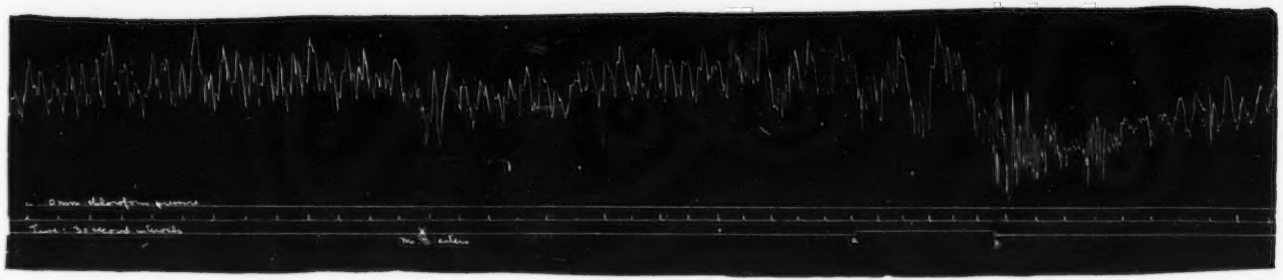

Fig. 3.

were increasing in vigor. There now occurred irregular twitchings of the toes and other muscles of the fore and hind limbs. As a result of these movements the more vigorous type of stomach contractions ceased. The dog woke up at "*b*" stretched herself and with the beginning of the purely sleeping state the stomach contractions gradually increased in vigor.

Figure 3. Dog sleeping in my lap showed vigorous type III hunger contractions. The tone of the empty stomach was temporarily inhibited at "*x*" when Mr. B. entered the room. The dog fell asleep and the tone of the stomach returned. Shortly before "*a*" on the tracing the respirations were deepened. From "*a*"–"*b*" the facial muscles contracted spasmodically, the feet moved, and the respiration was increased in depth. At "*b*" dog woke up with a start and this was followed by a complete cessation of the movements of the empty stomach which lasted for some time. It can be seen, however, that even before awakening the tone of the stomach was gradually diminishing.

DISCUSSION

Since the sensation of hunger, even when painful, does not itself inhibit further movements of the empty stomach the impulses which give rise to this inhibition must have their origin in some organ other than the stomach. The inhibitory impulses probably reach the stomach through the splanchnic nerves, the impulses having their origin in the central nervous system as one of the end effects of the emotional state. There is no evidence to support the view that the inhibition is brought about by the direct action of the splanchnic nerves on the stomach musculature or due to the increased output of adrenalin as Cannon and D. de la Paz have shown to be the cause of the inhibition of the movements of the stomach during digestion by fear, pain, rage.[3] The rapidity in the appearance of the inhibition points to nervous rather than to a chemical inhibition. It is possible but not probable that the inhibition results from afferent impulses arising from stimulation of the sensory nerve endings in the muscles, skin, and joints as a secondary effect of the active movements of the facial muscles and muscles of the extremities thrown into activity by impulses coming to them from the cerebrum.

[3] Cannon and de la Paz: This Journal, 1911, xxviii, 64.

SUMMARY

Although the contractions of the empty stomach (hunger contractions) become more vigorous with the onset of and during sleep because the inhibitory mechanism is no longer influenced by the sensory impulses reaching it through the optic, olfactory, and acoustic nerve (the extero-ceptive field of Sherrington) the hunger contractions may be greatly diminished even during sleep if the sleeping state is accompanied by pronounced cerebral activity (dreaming). The same cerebral state which effects movements of the limbs, tail, and muscles of the face effects an inhibition of the contractions of the stomach. The inhibition is therefore purely central in origin since it is not brought about by any impulse coming into the central nervous system by any known sensory nerve.

THE MODE OF ACTION OF ULTRA-VIOLET RADIATION IN INJURING LIVING CELLS WITH SPECIAL REFERENCE TO THOSE CONSTITUTING THE EYE

W. E. BURGE

Physiological Laboratory of the University of Illinois

(From experiments carried out at Nela Research Laboratory)

Received for publication November 29, 1915

Action on paramecia. Five cc. of water containing paramecia were introduced into a glass vessel 6 cm. in diameter and 4 cm. deep. This vessel was adjusted partially immersed in running water under a quartz mercury-vapor burner operating at 140 volts, 3.3 amperes, 2400 cp. so that the burner was 10 cm. from the surface of the liquid. The organisms were observed under a microscope during the exposure. These organisms are fairly transparent and appear to the unaided eye as white specks in the water. During the exposure the animals moved more and more slowly and gradually became granular and opaque. After 30 or 40 minutes they were dead. Figure I (1) represents the normal transparent animal, (2) represents the organism that was killed by ultra-violet radiation and (3) one killed by heating to 45°C. Just as exposure of egg white to ultra-violet radiation coagulates it and causes it to become an opaque mass, so a similar exposure causes the protoplasm or living material of these organisms to become coagulated and opaque. The conclusion may be drawn that ultra-violet radiation injures or kills living cells by coagulating or rendering insoluble the protoplasm or living material of the cells.

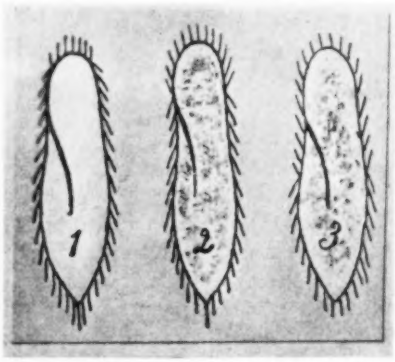

Fig. I. Paramecia. (1) The normal transparent animal. (2) Paramecium killed by ultra-violet radiation. (3) Paramecium killed by heating to 45°C.

Action on proteins. Egg white was poured on a glass plate 6 cm. square and permitted to dry. A piece of cardboard the size of the plate with a circle cut from its center approximately 2 cm. in diameter was fitted over the plate. The preparation was exposed for 30 hours to the radiation from the quartz mercury-vapor burner at a distance of 10 cm. It will be noted that only the central circular area of the egg white was exposed to the radiation since the peripheral portion was covered by the cardboard. At the end of the 30 hours' exposure the cardboard was removed and the plate was photographed.

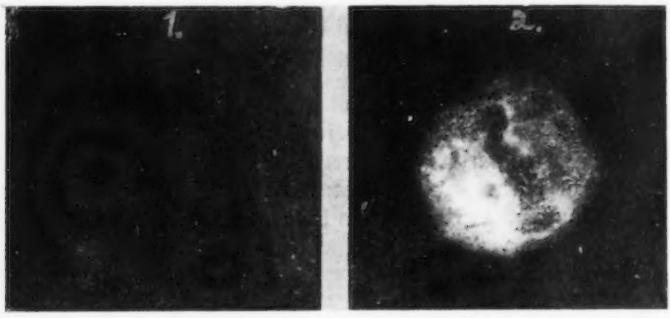

Fig. II. (1) is a photograph of the square of glass covered with egg white, the central area of which had been exposed to ultra-violet radiation. (2) is a photograph of the same square of glass after it had been immersed in a 0.1 per cent calcium chloride solution.

Figure II (1) is the photograph. It may be seen that at this time there was no apparent difference between the exposed central area and the unexposed peripheral area of the layer of egg white. The plate was then immersed in a 0.1 per cent calcium chloride solution. (2) is a photograph of the plate after it had been immersed in the solution for one hour. It may be seen that the calcium chloride precipitated the proteins of the egg white in the circular area where it had been exposed to the radiation, while it did not affect the egg white where it had not been exposed. Calcium nitrate was tried in the same manner and found to have a similar effect in precipitating the protein of the egg white previously exposed to the radiation.

A thin layer of lens material was made by pressing three fresh pig lenses between two quartz plates. The cardboard with a circular area cut from its center was placed over the preparation and this was ex-

posed to the radiation from the quartz mercury burner for 30 hours as the egg white had been. At the end of this time there was no apparent difference between the exposed circular area and the unexposed peripheral area of the lens material, both being transparent. When, however, the preparation was immersed, for about an hour in 0.1 per cent calcium chloride solution the exposed circular area became an opaque mass, while the unexposed peripheral area remained transparent. The conclusion may be drawn that ultra-violet radiation coagulates protein by changing it in such a way that salts such as those of calcium can combine with it to form a coagulum. In this respect it would seem that ultra-violet radiation acts on protein in very much the same way that certain enzymes act on it. It is known that rennin changes the protein in milk, caseinogen, in such a way that calcium salts can combine with it to form a coagulum.

Action of the different wave lengths in the ultra-violet region of the spectrum. An extract of twelve pig lenses was made with 25 cc. of 0.1 per cent calcium chloride solution and filtered through a coarse-grained filter. Five cc. of this fairly clear filtrate were introduced into a circular quartz cell made of two quartz discs having between them a ring of hard rubber 0.8 mm. thick with an inside diameter of 3.8 cm. By means of a small quartz spectrograph the radiation from a quartz mercury-vapor burner operating at 70 volts was focused on the lens extract in the quartz cell. The slit of the spectrograph was 1 mm. wide and the quartz burner was 3 cm. from the slit.

Figure III on following page (1) is a photograph of the spectrum that was focused on the lens extract. (2) is a photograph of the lens extract after the spectrum had been focused on it for 30 hours. The coagulated line of lens protein where the spectral line of wave length 254 $\mu\mu$ was focused, appeared after 50 minutes' exposure; that where the spectral line of wave length 265 $\mu\mu$ was focused appeared after 65 minutes' exposure; that where 280 $\mu\mu$ and 302 $\mu\mu$ were focused appeared after 120 minutes' exposure. The remaining lines of coagulated lens extract appeared after 200 minutes' exposure. (3) is a photograph of the spectrum made on a photographic plate with half the slit of the spectrograph covered with the cornea of a rabbit. It may be seen that the cornea transmits wave lengths as short as 297$\mu\mu$ and 302 $\mu\mu$ and in (2) it may be seen that these wave lengths are effective in changing the protein of the lens so that calcium salts can combine with it to form a coagulum. (4) is a photograph of the spectrum made through a layer of the lens extract 1 mm. thick. It may be seen that the extract absorbs all wave

lengths shorter than 313 $\mu\mu$ and in (2) it may be seen that all of these absorbed short wave lengths are effective in coagulating the protein of the lens extracted with 0.1 per cent calcium chloride solution. (5) is a photograph of the spectrum through a layer of aqueous humor 1 mm. thick.

In another experiment egg white was introduced into the same quartz cell that had been used with the lens extract. (8) is a photograph of the spectrum of the quartz mercury-vapor burner. (7) is a photograph of the region of the quartz cell containing the egg white where the spectrum had been focused for 30 hours. (6) is a photo-

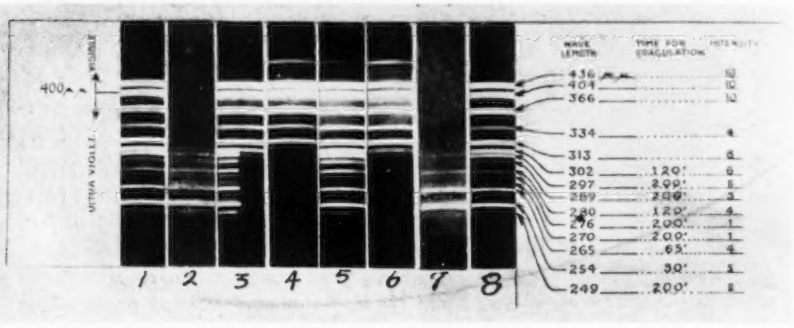

Fig. III. Photograph of spectra of small quartz mercury-vapor burner. (1) made on photographic plate; (2) made on lens protein; (3) made on photographic plate, one-half of the slit of the spectrograph being covered with the cornea of a rabbit; (4) through layer of lens portein 1 mm. thick; (5) through aqueous humor 1 mm. thick; (8) photograph of spectrum made on photographic plate; (7) made on egg white; (6) made on photographic plate through egg white 1 mm. thick.

graph of the spectrum through a layer of egg white 1 mm. thick. It may be seen that the egg white absorbs wave lengths shorter than 302 $\mu\mu$ and that all these absorbed wave lengths are effective in coagulating the proteins of the egg white. The periods of time required for the formation of the lines of coagulated egg white where the spectral lines were focused are indicated in figure III under "time for coagulation." It may be seen that the time required for the coagulation of egg white by the different spectral lines is the same as that required for the coagulation of the modified lens protein by the corresponding spectral lines.

The production of anterior eye trouble in living animals by means of ultra-violet radiation. One batch of six frogs was kept living partially immersed in 0.2 per cent sodium silicate, another batch in 0.8 per cent calcium chloride, another in 1 per cent dextrose, another in tap water for 15 days. At the end of this time one eye of each frog was exposed one hour each day for five successive days to the radiation from a quartz mercury burner operating at 140 volts, 3.3 amperes and 2400 cp. at a distance of 20 cm. Photographs of the frog were made 15 days after the exposures. Figure IV, frog (1) had been living partially immersed in tap water previous to the exposures. Frog (2) had been living partially immersed in 0.2 per cent solution of sodium silicate previous to the exposures. It may be seen that the eyelid of the frog living in the solution of sodium silicate had been converted into an opaque mass, while that of the frog living in tap water was very little injured. The solution of calcium chloride had the same effect as the solution of silicate. The dextrose was effective but not so much so as either of the other solutions. The conclusion may be drawn that salts such as are found to be greatly increased in human cataractous lenses increase the effectiveness of ultra-violet radiation in producing anterior eye trouble.

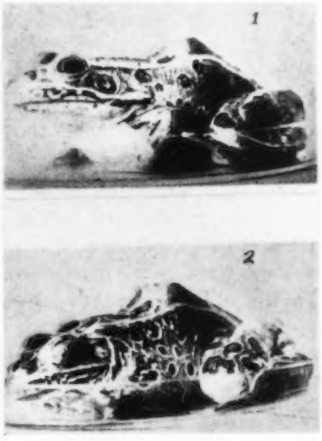

Fig. IV. Frog (1) living in tap water and exposed to ultra-violet radiation for 5 hours. Frog (2) living in 0.2 per cent sodium silicate and exposed to ultra-violet radiation for 5 hours.

It is a common experience that the skin sunburns more easily and quickly when it is wet than when it is dry. It is probable that if the skin is dry when it is exposed to sunlight, the ultra-violet radiation in the sunlight changes the protein of the cells of the skin in such a way that salts such as those of calcium in the lymph bathing the cells can combine with it to form a coagulum. If the skin is wet with ordinary fresh or salt water the salts in the water facilitate the process by combining with the proteins of the cells modified by the ultra-violet radiation.

The production of cataract in living animals by means of ultra-violet

radiation. An attempt was made to increase in the fluids of the body of living animals and hence in the eye media the salts found to be greatly increased in human cataractous lenses with the hope that on exposure of the eyes of these animals to ultra-violet radiation cataract would develop. Many observers have demonstrated that it is impossible to produce an opacity of the lens or cataract in the normal living animal by exposure of its eyes to ultra-violet radiation. Moreover, it has been shown that it is not possible to produce an opacity even in excised pig lenses by exposing these directly to the radiation from a quartz mercury-vapor burner for very long periods of time. Fish were chosen for the experiments to be described because they could be kept alive in the solutions of the salts desired. One batch of gold fish was kept in 0.8 per cent calcium chloride solution, another in 0.8 per cent calcium nitrate solution, another in 1 per cent dextrose, another in 0.1 per cent sodium silicate for ten days. At the end of this time each fish in its turn was introduced into a small box with a quartz window in one side. In practice four of these boxes were used so that one eye of each of the four fish was exposed simultaneously. The boxes containing the fish were adjusted so that the quartz windows were 15 cm. from the quartz mercury-vapor burner operating at 140 volts, 3.3 amperes, and 2400 cp. In this manner one eye of each fish was exposed to the radiation. Each exposure was of 6 hours' duration. After the exposure, the batches of fish were replaced in the solutions from which they had been removed. For comparison the eyes of fish living in tap water were exposed in the same manner and for a similar length of time as those living in the salt solutions. As a rule a slight opacity in the cornea of the eye exposed appeared about 15 hours after the first exposure. It was assumed that this opacity did not develop during the exposure because there was not sufficient salt present in the cells of the cornea to combine with the protein modified by the radiation to form a coagulum. However, at the end of 12 or 15 hours a sufficient quantity of salt had collected, owing to the diffusion of the salt from the blood stream into the cornea, and had combined with the modified protein to precipitate it and hence an opacity of the cornea was produced. This assumption would seem to explain the so-called "latent period," i.e., the time elapsing between the exposure of one's eye to ultra-violet radiation and the time when the painful effect is felt. In most of the fish a slight clouding appeared about 2 days after the first exposure in the lens of the eye exposed.

Ten days after the first exposure the eyes of the fish that had been

used were exposed again for another 6 hour period. At the time of this second exposure as a rule an opacity of the cornea and of the lens of the fish living in the salt solutions had increased while the opacity of the cornea of the fish living in tap water had practically cleared up. Several hours after the second exposure as a rule the opacity of the lens and of the cornea of the fish living in the salt solutions became more marked. An opacity of the cornea of the fish living in tap water developed also but this was slight and cleared up in a few days while that of the fish living in the salt solutions increased.

After the second exposure no prescribed rule as to time for the third exposure can be laid down. In order to clear up the opacity of the cornea of the fish in the different salt solutions it was necessary to transfer them to tap water. As a rule the opacity of the cornea cleared up

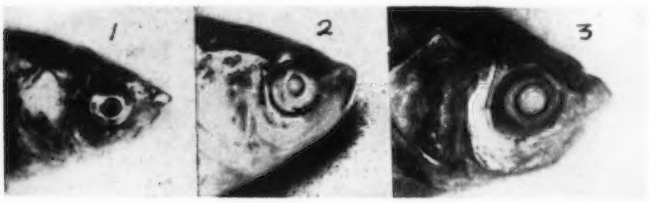

Fig. V. Fish (1) living in tap water and exposed to ultra-violet radiation for 12 hours. Fish (2) living in 0.1 per cent sodium silicate and exposed to ultra-violet radiation for 12 hours. Fish (3) living in 0.1 sodium silicate and exposed to ultra-violet radiation for 24 hours.

in a few days while the lens remained opaque. By nursing, by exposing to ultra-violet radiation, by transferring back and forth from salt solution to tap water it was possible to obtain fish in the condition indicated in figure V. Fish (1) had been living in tap water for 30 days and had been exposed to ultra-violet radiation for two 6 hour periods or 12 hours. Fish (2) had been living in 0.1 per cent sodium silicate for 28 days and had been exposed to ultra-violet radiation for two 6 hour periods or 12 hours. Fish (3) had been living in 0.1 per cent sodium silicate for 42 days and had been exposed to ultra-violet radiation for four 6 hour periods or 24 hours.

It may be seen that the lens of fish (3) living in the silicate solution and exposed to ultra-violet radiation for 24 hours had become perfectly opaque; that of fish (2) living in the same solution but exposed to ultra-violet radiation for 12 hours had become partially opaque while the

lens of fish (1) living in tap water and exposed to ultra-violet radiation for 12 hours was practically clear.

In figure VI are shown the photographs of the spectrum of the small quartz mercury-vapor burner operating at 70 volts and that of a large quartz mercury-vapor burner operating at 140 volts. Photographs showing the effects produced by focusing these spectra on egg white and on lens protein are also given in order to show that the most effective region of the two spectra in precipitating protein are different. (1) is the photograph of the spectrum of the small quartz mercury-vapor burner made on a photographic plate, (9) is a photograph of the spec-

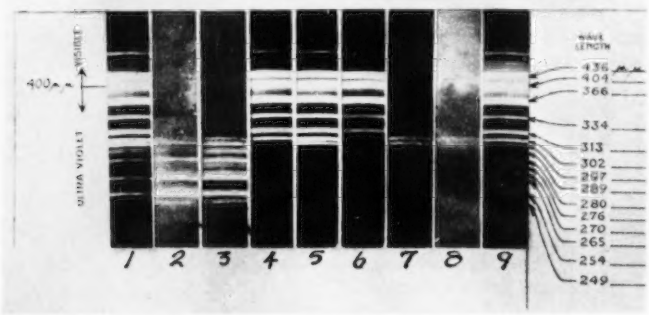

Fig. VI. Photograph of spectra of the small quartz mercury-vapor burner and of the large quartz mercury-vapor burner. (1) That of the small burner; (9) that of of the large burner, made on a photographic plate; (2) that of the small burner; (8) that of the large burner, made on egg white; (3) that of the small burner; (7) that of the large burner, made on lens protein extracted with 0.1 per cent calcium chloride solution; (4) that of the large burner made through cornea of the rabbit; (5) made through glass 1 mm. thick; (6) through glass 5 mm. thick.

trum of the large quartz mercury-vapor burner made on a photograpic plate. By comparing these two spectrograms it may be seen that the spectral lines in the extreme ultra-violet region of the spectrum are much more intense in (1) than they are in (9). (2) is a photograph of the cell containing egg white on which the spectrum of the small burner had been focused for 30 hours. (8) is a photograph of the quartz cell containing egg white on which the spectrum of the large burner had been focused for the same length of time. By comparing (2) and (8) it may be seen that the egg white was coagulated in (2) where the spectral lines of wave lengths 302 $\mu\mu$ to 249 $\mu\mu$ were focused, while in

(8) the egg white was coagulated only where the spectral lines of wave lengths 302 $\mu\mu$ and 297 $\mu\mu$ were focused. (3) is a photograph of the quartz cell containing lens protein extracted with 0.1 per cent calcium chloride solution on which the spectrum of the small burner had been focused for 30 hours, (7) is a photograph of the quartz cell containing the same kind of extract on which the spectrum of the large burner had been focused for 30 hours. By comparing (3) with (2) and (7) with (8) it may be seen that the same region of the spectrum of the small quartz mercury-vapor burner effective in coagulating the proteins of egg white is also effective in coagulating the lens protein extracted with 0.1 per cent calcium chloride solution. The same statement may be made regarding the effective region of the large quartz mercury-vapor burner in (7) and (8). (4) is a photograph of the spectrum of the large quartz mercury-vapor burner made through the cornea of the rabbit, (5) made through glass 1 mm. thick, (6) through glass 5 mm. thick. It may be seen from (4) that the cornea transmits wave lengths as short as 297 $\mu\mu$ and 302 $\mu\mu$ and from (7) that these are the wave lengths of the spectrum of the large quartz mercury-vapor burner most effective in bringing about the coagulation of the protein of the lens. The large quartz mercury-vapor burner was the one used in the experiments described in which cataract was produced in fish living in salt solutions.

Many observers have attributed their failure to produce cataract in living animals to the protective action of the cornea. I think it will be clear from the experiments reported in this paper that their failure was not due to the protective action of the cornea but to the fact that there were not present in the lens of the eyes of the animals exposed sufficient quantities of salts such as those of calcium to combine with the protein of the lens modified by the ultra-violet radiation to precipitate it. It has been shown that the quantity of calcium in the normal lens is less than 0.08 per cent of the ash and that the amount in human cataractous lenses is about 15 per cent of the ash.

Cataract is of common occurrence among people living in the tropics. Analyses of human cataractous lenses from India show that they contain large quantities of calcium salts and silicates. No silicates were found in human cataractous lenses from the United States. Silicates may be accounted for in the cataractous lenses from India by the fact that silicous earths form a part of the diet of certain classes in India, while the prevalence of cataract in India may be attributed possibly to the comparatively large amount of ultra-violet radiation in

ordinary tropical light and the silicates present in the eye media. The assumption is made that the ultra-violet radiation modifies the protein of the lens so that the silicates can combine with this modified lens protein to precipitate it and hence form an opacity of the lens or cataract.

Cataract is of comparatively frequent occurrence among glass blowers. The eyes of glass blowers are probably subjected to more of the short wave lengths of the spectrum than are the eyes of people generally. It may be assumed that these short waves keep the protein of the lens modified so that if abnormal amounts of the salts of calcium or silicates, etc., are present these combine with the modified lens protein to precipitate it and hence produce an opacity of the lens. Not all glass blowers develop cataract although their eyes are subjected to the same quantity and quality of radiation from the furnaces. It is assumed that those who do develop cataract have a more or less disturbed condition of nutrition expressing itself in an increase of those substances which can combine with the lens protein, modified by ultra-violet radiation, to precipitate it.

CONCLUSIONS

1. Ultra-violet radiation kills living cells and tissues by changing the protoplasm of the cells in such a way that certain salts can combine with the protoplasm to form an insoluble compound or coagulum. The effective region of the spectrum in changing the living material of the cell or protoplasm lies between 254 $\mu\mu$ and 302 $\mu\mu$. The most effective region is around 254 $\mu\mu$ for the small quartz mercury burner used and around 302 $\mu\mu$ for the large quartz mercury burner used.

2. An opacity of the lens or cataract can be produced in fish living in solutions of those salts found to be greatly increased in human cataractous lenses by exposing the eye of the fish to radiation from a quartz mercury-vapor burner. This cannot be done by exposing the eyes of fish living in tap water containing very small quantities of these salts.

3. Abnormal quantities of the salts of calcium and sodium silicate in the cells of the eyelids and of the cornea increase the effectiveness of ultra-violet radiation in producing anterior eye trouble. Abnormal quantities of calcium salts on the skin also increase the effectiveness of the short wave lengths in sunlight in producing sunburn.

THE EFFECTS UPON THE GASTRIC SECRETION OF ORGAN EXTRACTS

JOHN ROGERS, M.D., JESSIE M. RAHE, M.A., GEORGE G. FAWCETT, M.D., GEORGE S. HACKETT

From the Johnston Livingston Fund for Experimental Medicine in the Department of Medicine, Cornell University Medical College

Received for publication December 1, 1915

This publication is the fourth in our series upon the physiology of the ductless glands. The first[1] dealt with the effects upon blood pressure of the different substances which can be isolated, without material alteration of their chemical structure, from an aqueous extract of the thyroid gland. We demonstrated in this article that the "residue," or non-coagulable portion, of the extract was the only portion which produced a fall in blood pressure. The second article[2] dealt with the blood pressure effects of all the similar substances which can be isolated from other organs. In this we demonstrated that the "residue," or non-coagulable portion, of the aqueous extract of all organs (except the adrenal) is the only portion which contains the depressor principle. The adrenal "residue" contains a pressor substance which shows certain marked differences in its effects in the kymograph tracings from those produced by the 1:1000 solution of commercial adrenalin.

When the dose of "residue" is standardized according to its nitrogen content, the residue of each organ produces a characteristic effect in the kymograph tracings. It is, therefore, reasonable to infer that the depressor substance in these residues is peculiar to and characteristic of the organ from which it is derived. The "basic principle" of these depressor substances, which means some particular portion of its molecule, may be the same but the molecule which contains the depressor principle also contains some other molecule or atom or ion, and this addition to the basic depressor principle constitutes an active principle which can be isolated from every organ and is characteristic of and peculiar to that organ.

[1] Fawcett, Rogers, Rahe and Beebe: This Journal, 1915, xxxvi, 113.
[2] Fawcett, Rogers, Rahe and Beebe: This Journal, 1915, xxxvii, 453.

The term "active principle" is used to signify a substance which can produce some one or more demonstrable physiological effects. In this sense every organ seems to contain at least one active principle, and this differs from that of every other organ.

The kymograph tracings seemed to prove that this active principle of each organ was to be found only in the non-coagulable, or "residue," portion of its aqueous extract. To confirm these observations we next tested the effects of the different bodies which can be isolated from aqueous extracts of organs, upon the contractions of unstriated muscle fiber. In this third report[3] we demonstrated that the "residue" or non-coagulable portion of our extracts and no other portion: (a) induced contractions, (b) that these contractions appeared to be characteristic for each "residue," (c) that the peculiar contractions induced by each residue were paralyzed by the subsequent addition of a few drops of a 1:1000 solution of adrenalin. It therefore seemed reasonable to conclude that the active principle in each residue exerted its effects through the terminal filaments or the end plate or the intermediate substance of the distal extremity of the sympathetic nerves. To confirm and elaborate these observations we next tested the effects of our residues upon the secretory activity of readily accessible organs. This fourth report contains the results as regards the stomach.

It is well known that extracts of many kinds made from many different tissues and organs when administered by hypodermic injections affect the flow of gastric juice and also its pepsin and hydrochloric acid content. A recent article on this subject[4] signifies the material or substance which stimulates the gastric secretion as "gastrin." This suggests the presence, particularly in the epithelial portions of the digestive tract, of a body which has more or less of a specific hormone action upon the secretions of the stomach. But the increase of gastric secretion can be produced by too many substances to warrant the assumption of any hormone or "gastrin" body which acts only upon the stomach. The stimulant action seems to be of a more general character. Although its exact nature can as yet only be surmised, we suggest, from our experiments, that the equivalent of a gastric hormone or gastric stimulant is to be sought in some substance common to many extracts which affects favorably the nutrition and consequently the activity of the gastric epithelial cells and has some relation to their nerve supply.

[3] Fawcett, Rahe, Hackett and Rogers: This Journal, 1915, xxxix, 154.

[4] Keeton and Koch: This Journal, 1915, xxxvii, 481.

In our tests of the effects of organ extracts upon the gastric juice, we employed dogs in which had been established the Pawlow isolated stomach pouch and fistula. These animals were placed in a retaining frame to which they quickly became accustomed and remained quiet for two or three hours, or a long enough period to permit accurate estimations of the flow from the gastric pouch of its secretion. The size of this pouch, of course, determines the quantity of the secretion and the results are, therefore, relative in each animal. The materials tested were standardized according to their nitrogen content, as in the previous kymograph and muscle experiments. They were administered with every aseptic precaution in measured doses with a hypodermic needle into the subcutaneous tissue of the back or flanks. In our experiments upon blood pressure[5] it was ascertained that these organ extracts when thus injected subcutaneously even in enormous doses, showed no vaso-dilating effect. Hence it is not at all probable that any increase of the gastric secretion consequent upon the subcutaneous injection of our organ extracts can be caused by splanchnic dilatation. The dogs received no food for at least twelve hours before the tests, and immediately after their conclusion were not rewarded with food. All psychic stimuli were thus carefully avoided. The normal fasting flow during a period of fifteen to thirty minutes was determined before each test. After every injection there was found to be a "latent" period in the flow of gastric juice, lasting about ten minutes. The increase, if it occurred appeared quite suddenly and continued for from one and a half to three hours. This corresponds fairly closely to the results which followed the administration by mouth of test meals of milk and bread, or meat. The total acidity of the collected secretion was determined by titration against $\frac{N}{10}$ NaOH with phenolphthalein as the indicator. The free acid was determined by titration against dimethyl-amido-azo-benzine. Both results are expressed in terms of HCl.

In the first experiments the materials tested consisted of definite and comparable amounts of the substances which can be isolated from an aqueous extract of the pig thyroid gland. These substances in all probability closely approximate, chemically, those which presumably exist during life. They are the nucleoproteins, and the globulins. The filtrate or "residue" which remains after the removal of these bodies and the coagulable proteins, is evaporated to dryness, and is

[5] **Fawcett, Rogers, Rahe and Beebe: This Journal, 1915, xxxvii, 121.**

then divisible into an alcohol soluble and an alcohol insoluble substance. The alcohol soluble part can be still further subdivided by the use of basic lead acetate into fractions which we have called, for the sake of convenience, a "lead precipitate" and a "lead filtrate." This "lead filtrate" seemed to contain in our first series of experiments nearly if not all of the active material in the thyroid and other organs. The following tables, I and II, show the results of these tests upon the same dog. They were repeated upon several other animals with approximately the same findings and apparently confirm the localization of an active principle obtainable from the thyroid gland in the "residue" or non-coagulable portion of its extract, and in the particular subdivision of this residue which we have designated the "lead filtrate" from the alcohol soluble part of the original residue.

This final filtrate and, of course, the preceding alcoholic solution, contains a substance which shows a pronounced stimulating effect upon the flow of gastric juice and upon its content of hydrochloric acid. Tests which are not recorded in the table, also show that this increase in quantity and in hydrochloric acid are accompanied by an increase in peptonizing power. The whole "residue" of the thyroid seemed, however, somewhat more active than any of its active fractions.

After testing the effects upon gastric secretion of the thyroid residue, a similar extract was made from muscle tissue. The effects of the subcutaneous injection of the muscle residue and of Witte's peptone and of the ingestion by mouth of milk and beef heart are recorded in the first three lines of Table II. These are entirely negative.

In this same table there are recorded in detail the effects of the injection of the "residues" of the liver, pancreas and spleen and of the pituitary, parathyroid, thymus, pineal and adrenal glands.

The residues of the pituitary, pineal, adrenal and thymus glands and of muscle tissue, as well as Witte's peptone, are practically inert. The other residues are very active gastric stimulants.

We have repeated the tests of effects of extracts made from the beef pituitary (whole gland), the beef pineal and parathyroid, thymus, liver, pancreas, spleen and adrenal glands, and recorded the results in Table III. As the entire thyroid residue seemed a little more active than its fractions, we employed the entire "residue" or non-coagulable portion of extracts of these other organs and compared the effects with those produced by a solution of the combined nucleo-proteins, globulins and coagulable proteins. Except in the case of the pancreas, the coagulable portion of these extracts were uniformly inactive. At the

TABLE

TESTED PORTION OF AQUEOUS EXTRACTION OF THYROID	AMOUNT INJECTED EXPRESSED IN MGM. OF PROTEIN	NORMAL COLLECTION FROM PAWLOW FISTULA			SECRETION COLLECTED AFTER INJECTION DURING PERIODS OF 20 MINUTES											
					First 20 mins.			Second 20 mins.			Third 20 mins.			Fourth 20 mins.		
		Time and amount	Total acid	Free acid	Amt.	Total acid	Free acid	Amt.	Total acid	Free acid	Amt.	Total acid	Free acid	Amt.	Total acid	Free acid
			per cent	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*
Nucleo-proteins	30 cc. 600 mgm.	25 min. 2.5 cc.	0.2		2	0.2		0.25			1	0.2		0.25		
Globulins	40 cc. 600 mgm.	20 min. 2 cc.	0.28		2	0.16		1	0.17		2	0.12				
Alcohol insol. part of residue	20 cc. 600 mgm.	20 min. 1 cc.	0.1		0.25			1	0.1		1	0.1				
Lead precipitate from alc. sol. part of residue	50 cc. 600 mgm.	25 min. 1 cc.	0.12		1.5	0.12		0.5			1	0.12				
Residue	20 cc. 620 mgm.	30 min. 1 cc.	0.24	0.08	6	0.52	0.36	9	0.6	0.44	10	0.64	0.48	12	0.6	0.44
Lead filtrate from alc. sol. part of residue	20 cc. 600 mgm.	20 min. 1 cc.	0.28		5	0.28		8	0.34		7	0.55		3	0.52	
Column number		2			5			8			11					

Compare Column No. 2 with Columns Nos. 5, 8 and 11 to perceive at a glance the effect of the injected material upon the quantity of gastric secretion.

TABLE II

MATERIAL TESTED	AMOUNT INJECTED (CUT) IN MGM. OF PROTEIN	NORMAL COLLECTION FROM PAWLOW FISTULA			COLLECTION FROM PAWLOW FISTULA AFTER INJECTION RESULTS GIVEN IN 20 MINUTE PERIODS											
					First 20 mins.			Second 20 mins.			Third 20 mins.			Fourth 20 mins.		
		Time and amount	Total acid	Free acid	Amt.	Total acid	Free acid	Amt.	Total acid	Free acid	Amt.	Total acid	Free acid	Amt.	Total acid	Free acid
			per cent	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*
Muscle residue	20 cc. 600 mgm.	20 min. 1 cc.	0.32		1.25	0.32		1.5	0.34		0.5			1	0.34	
Witte's peptone	40 cc. 600 mgm.	20 min. 1.5 cc.	0.2		0.5	0.2		0			Total of 2 and 3, 1 cc.	0.2				
300 cc. milk plus 250 gm. beef heart by mouth		20 min. 1 cc.	0.28	0.08	1	0.36		5	0.48	0.32	5	0.48	0.32	4	0.44	0.28
Pituitary residue (entire gland)	25 cc. 600 mgm.	20 min. 0.4 cc.	0.5	0	0.6	0.7	0	0.4	0.7	0	0.24	0.9	0	0.4	0.7	0
Parathyroid residue	35 cc. 600 mgm.	35 min. 0			4.5	0.56	0.36	10	0.6	0.48	10	0.64	0.48	8	0.64	0.48
Thymus residue	20 cc. 600 mgm.	20 min. 1 cc.	0.28	0.08	2.5	0.48	0.28	2.5	0.48	0.28	1	0.44	0.2			
Spleen residue	20 cc. 600 mgm.	25 min. 1 cc.	0.44	0.24	9	0.6	0.4	13	0.68	0.48	13	0.72	0.52	10	0.68	0.44
Liver residue	20 cc. 600 mgm.	25 min. 1 cc.	0.24	0.12	5.5	0.6	0.4	7	0.6	0.4	5	0.6	0.4			
Adrenal residue	15 cc. 600 mgm.	25 min. 1 cc.	0.44	0.28	1	0.4	0.24	0			Total of 2 and 3 1.25 cc.	0.24	0.12			
Pineal residue	40 cc. 600 mgm.	25 min. 1 cc.	0.4	0.16	3.5	0.52	0.36	1.5	0.52	0.28	0			0		
Pancreas residue	6 cc. 600 mgm.	35 min. 1.25 cc.	0.28	0.08	10	0.6	0.44	12	0.72	0.56	13	0.68	0.52	10	0.6	0.44
Column number		2			5			8			11					

Compare Column No. 2 with Columns 5, 8 and 11 to perceive at a glance the effects of the different residues upon the quantity of secretion.

conclusion of the tests the pneumogastric nerves in two of the animals were cut through a transthoracic operation. From one to two inches of the nerves were excised just above the diaphragm, as it was found by experiment that simple section was quickly repaired. Two weeks later, or after the wounds had healed and distal degeneration of the nerves had presumably occurred, the tests with the gastric stimulant residues were repeated. There was little or no change in the stimulant effect.

The results recorded in the third table are portrayed less elaborately than the first. The tabulation represents the amount of normal flow with its free and total acid content from the gastric fistula in a fasting dog for the thirty minutes preceding the test injection, and for the second thirty minutes following the injection. Six dogs were employed. Numbers 5 b and 6 b record the results after resection of the thoracic portions of the pneumogastric nerve. In the next to the last line in the first column of the table there occurs the term "desiccated thyroid residue." This refers to the "residue" part of an extract made from a desiccated commercial thyroid powder. The commercial tablets or powder are crude and often unreliable medicaments. These tests, as well as some previously made upon blood pressure, seem to indicate that dessication of the entire gland may destroy much or all of its potency or active principle.

In these, as in the first experiments, the thyroid "residue," or the non-coagulable portion of its aqueous extract, is proved to contain an active stimulant for both the total quantity and the acid content of the gastric secretion.

The liver "residue," or non-coagulable portion of an aqueous extract of the liver, and similar residues of the parathyroid, the spleen and the pancreas are even more active gastric stimulants. Unlike all the others, the coagulable portion of a pancreatic extract was equally active or more active than the pancreatic residue. We can as yet offer no explanation for the reasons of this peculiarity of the pancreas. The pituitary residue and the adrenal residue seemed to inhibit the gastric secretion. The thymus residue in some dogs was a mild stimulant, in others negative. The pineal residue was inactive.

We have been particularly interested in the effects of the thyroid residue and have observed that it not only stimulates gastric secretion but also gastric motility. After opening the abdomen of an animal and observing the contractions of the stomach, we have then injected subcutaneously the thyroid residue. Shortly afterwards there

TABLE III

MATERIALS TESTED	No. 1 PAWLOW STOMACH			No. 2 PAWLOW STOMACH			No. 3 PAWLOW STOMACH			No. 4 PAWLOW STOMACH			No. 5-a PAWLOW STOMACH			No. 5-b PAWLOW STOMACH X NERVE CUT			No. 6-a PAWLOW STOMACH			No. 6-b PAWLOW STOMACH X NERVE CUT		
	Flow in 30 min.	Free HCl per cc.	Total acid per cc.	Flow in 30 min.	Free HCl per cc.	Total acid per cc.	Flow in 30 min.	Free HCl per cc.	Total acid per cc.	Flow in 30 min.	Free HCl per cc.	Total acid per cc.	Flow in 30 min.	Free HCl per cc.	Total acid per cc.	Flow in 30 min.	Free HCl per cc.	Total acid per cc.	Flow in 30 min.	Free HCl per cc.	Total acid per cc.	Flow in 30 min.	Free HCl per cc.	Total acid per cc.
Normal flow	0.5	0	0.6	1.5	0.2	0.6	1.8	0	0.9	1.0		0.3	8.0	0.9	1.3	0.3	0.5	1.0	18.0	1.8	2.3	0.3	0	0.5
Thyroid residue—Subcutaneously	2.0	0.8	1.2	18.0	1.1	1.5	18.0	0.6	1.5	11.0		1.6	16.4	1.0	1.5	1.8	1.0	4.5	28.5	1.9	2.4	8.0	1.7	2.4
Normal flow	0.4	0	0.6				0			1.0		0.7							10.6	0.7	1.0	0.1	0.5	1.0
Thyroid coagulables	0.4	0	0.6				0			1.0		0.4							0.8	1.2	2.0	0.2	0.5	1.5
Normal flow	0.6	0	0.3	1.5	0.3	0.6				1.0		0.7	4.2	1.0	1.2	0.4	0.6	3.7	4.4	0.6	1.6			
Liver residue	2.0	0.3	1.5	10.5	1.0	1.5				9.0		1.1	15.0	1.0	1.6	0.7	0.7	4.0	8.4	0.8	2.2			
Normal flow	0.3	0	1.5																					
Liver coagulables	0.3	1.0	2.5																					
Normal flow	0.3	0	1.5	0																				
Parathyroid residue	3.0	1.5	1.7	15.0	1.2	1.6																		
Normal flow	0.6	0	0.7	0																				
Pancreas residue	6.0	0.9	2.2	7.2	0.5	1.3																		
Normal	0.4	0	0.5	1.0	0.3	0.8																0.6	0.8	1.1
Pituitary residue	0.6	0	0.7	0.5	0.1	0.5																0.0	0	0
Normal	0.3	0	2.0	1.2	0.2	0.7																		
Pancreas coagulables	5.1	1.9	2.2	19.5	1.4	1.8																		
Normal	0.3	0	1.5	1.2	0.2	0.7																		
Thymus residue	7.5	1.4	2.1	4.0	0.7	1.2																		
Normal	0.4	0	0.6																					
Thymus coagulables	0.4	0	1.0																					
Normal	0.7	0.7	2.0	1.5	0.7	1.1	0.6																	
Adrenal residue	0.5	1.0	2.5	1.0	0.3	0.6	0.3																	
Normal	0.4	0	1.1	1.5	0.6	1.1																		
Spleen residue	5.2	1.0	1.1	19.5	1.3	1.8																		
Normal																						0.6	0	0.7
Desiccated thyroid residue																						2.0	0.5	1.3
Normal				1.5	0.2	0.7	4.0		0.7															
Food				7.5	0.8	1.2	27.0		1.6										5.0	0.9	1.0			

'Res.' or 'Residue' refers to the non-coagulable portion of the aqueous extract of an organ.
'Coags.' or 'coagulables' refers to the substances in solution in the remaining part of the extract.

regularly occur in the stomach very vigorous peristaltic waves. These continue for a much shorter time than the increased secretion, but they regularly follow the injection of even small amounts of the thyroid residue. Sometime in the future we hope to depict this interesting phenomenon graphically. Thus far our attempts have been rather unsatisfactory. The residues show no change in their stimulating effects upon gastric secretion after resection and degeneration of the pneumogastric nerves.

Pawlow has demonstrated the powerful stimulant effect of the tenth cranial nerve upon gastric secretion. It is presumable that all of these stimulant residues act through some peripheral mechanism. From our previous experiments, this can be inferred to be either purely nervous or a combination of the nervous, cellular, and vascular elements.

CONCLUSIONS

1. The thyroid, or only the non-coagulable portion of an aqueous extract of the thyroid, contains a substance which is an active stimulant of both the gastric secretion and the gastric motility.

2. Only the residue portion of an aqueous extract of the parathyroid and thymus glands, and of the spleen and liver, have a similar effect.

3. Both the coagulable and the non-coagulable portions of an aqueous extract of the pancreas are very vigorous stimulants of gastric secretion.

4. The pituitary and adrenal residues inhibit the flow of gastric secretion.

5. All the stimulant residues seem to act upon some peripheral gastric mechanism in which the nervous system is an essential part.

6. The residue, or non-coagulable portion of an aqueous extract of the pituitary, pineal, thyroid, parathyroid, thymus and adrenal glands, and of the spleen and liver, contains all of the material which shows a demonstrable action upon other organs.

Res. or Residue refers to the non-coagulable portion of the aqueous extract of an organ.
'Coags.' or 'coagulables' refers to the substances in solution in the remaining part of the extract.